A STUDY ON URINARY CYTOLOGY WITH HISTOPATHOLOGICAL CORRELATION IN LESIONS OF URINARY BLADDER

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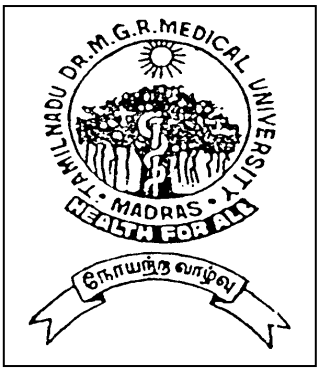

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CERTIFICATE

This is to certify that the dissertation entitled **A STUDY ON URINARY CYTOLOGY WITH HISTOPATHOLOGICAL CORRELATION IN LESIONS OF URINARY BLADDER** submitted by **Dr. M.SUMATHI** to the Faculty of Pathology, The Tamilnadu Dr. M.G.R. Medical university, Chennai in partial fulfillment of the requirement for the award of M.D. Degree in Pathology is a bonafide work carried out by her during the period September 2006 – August 2008 under my direct supervision and guidance.

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CONTENTS

INTRODUCTION

Cytologic examination of a urine specimen is a simple, safe, and inexpensive method that may uncover a hidden urothelial cancer. Since the entire mucosal surface, including the farthest reaches of the urinary tract, is bathed in this easily obtainable fluid, in theory, urine is the perfect specimen to examine for evidence of tumor.

Urine cytology is primarily used for diagnosis of symptomatic patients, detection of cancer in high-risk patients (eg, those exposed to industrial chemicals and metals, cigarette smokers, and those with schistosomiasis), and follow-up of patients with history of urinary tract neoplasia. It complements, but does not replace, cystoscopy and biopsy. However, lesions may be detected cytologically before they can be seen cystoscopically. Urinary cytologic examination is capable of detecting small or hidden lesions (eg, in diverticuli).

Urinary cytology can detect most aggressive neoplasms as well as carcinoma in situ. Patients with low-grade noninvasive tumors can be followed up cytologically. Patients with negative cytologic findings have a very low risk of recurrence, while high-grade cytologic abnormalities predict an aggressive tumor course (Harving 1989).

As the incidence of urothelial carcinoma increases, so too does the demand for urine cytology. Accuracy of diagnosis is always important, but conservative management of bladder cancer depends on accurate cytodiagnosis. Clinical history is imperative for the reduction of misdiagnoses.

A variety of newer diagnostic techniques, including flow cytometry, image analysis/quantitative cytology, cytogenetics, immunology (eg, blood group isoantigens and monoclonal antibodies to a variety of tumor-associated antigens) and molecular biology have been studied in an effort to increase diagnostic accuracy. However, at least for the moment, urinary cytopathologic examination remains one of the most clinically useful means of diagnosing urothelial cancer and predicting its biologic behavior and hence this work is taken up.

AIM OF STUDY

- To evaluate urinary cytology of the patients with history of hematuria, dysuria and frequency of micturition, coming to the urology department of Government Rajaji Hospital and Meenakshi Mission Research Centre, Madurai.

- To correlate the cytological findings with histopathological diagnosis.

- To assess the usefulness of the cytological study in the diagnosis of urinary bladder lesions.

REVIEW OF LITERATURE

The earliest mention of the use of urine cytology, for the diagnosis of bladder cancer is Sander's report of finding neoplastic tissue in urine in 1864.

The results of cytological examination of urinary sediment for diagnosis of urinary tract carcinomas were later published by Papanicolaou in 1945 and the procedure is now established as part of the routine investigation of patients with hematuria,prostatism and suspected urinary tract neoplasia.

Park CH et al (1969) described that ideally at least 3 urine specimens should be examined.

Geisse LJ et al (1978) found that the sensitivity of a single voided specimen for bladder neoplasia was 97% in one report, rising to 100% when two or more specimens were examined.

Trott PA et al (1973) described in their study that the cellular yield and preservation of the bladder washings were usually good, making this method of collection superior to voided urine.

Matzkin et al (1992) postulated that the sensitivity for detection of neoplasia in one reported study was 100% for bladder washings.

Farrow GM (1990) had described that direct smears were easier to prepare but showed scanty cellularity.

Rife CC et al (1979) said that the larger the tumour the greater the likely hood of malignant cells being found in urine, but the relationship between the number of tumours present and cytological evidence of neoplasia is not as reliable.

Kannan V (1990) postulated that positive urinary cytology could also be due to urothelial tumours of the upper urinary tract rather than in the bladder. These constitute 5-8% of all urothelial cancers.

Murphy WM (1990) described that only 30-60% of grade I urothelial carcinomas exfoliate carcinoma cells.

Fontana P et al (1993) described that Bizarre spindle cells may be seen in urine from patients with low grade papillary carcinoma.

Koss LG (1979) described that, invasive carcinomas were especially prone to develop from flat abnormalities such as atypical hyperplasia and carcinoma in situ.

Shenoy UA et al (1985) postulated that carcinoma in situ is diagnosed as a grade III transitional cell carcinoma in 50% of cases, because the cells exfoliated in carcinoma in situ show features such as pleomorphism suggestive of high grade malignancy ,similar to those seen in biopsy specimens.

Colby TV et al (1985) stated that urothelial grade III carcinoma with marked plemorphism was found with some syncytial formations and “cell- in a-cell” arrangements referred to as cannibalism.

Cotran R et al (1989) described that 55% of patients with flat carcinoma in situ lesions develop invasive carcinoma with in 5 years.

Highman W et al (1982) found that there were two types of papillary clusters seen in calculi, a smooth-bordered type and irregular clusters of cells with ragged borders and abnormal nuclei. The later which were seen in a minority cases showed features similar to those of a low grade urothelial carcinoma.

Kannan V et al (1993) found a helpful feature of cell clusters dislodged by catheterization is the presence of smooth outer contour, in a contradiction to the ragged or irregular margins seen in papillary clusters from well differentiated urothelial tumours.

Kupper T et al (1993) in their study described that viral induced cytopathological changes in urine should not be mistaken for malignancy since the nuclear changes are similar to those of high grade urothelial neoplasia .

Normal anatomy

The urinary bladder is the epithelial lined muscular viscus that can distend and hold up to 400-500 ml of urine without a change in intraluminal pressure. It is a hallow viscus resembling inverted pyramid when empty and sphere when distended. Has superior surface (apex, dome), posterior surface (base) and inferolateral surfaces. Trigone is area between ureteral and urethral

orifices, continuous with bladder neck. Bladder rests on rectum and seminal vesicles (males) or cervix and vagina (females)

Embryology

Bladder develops during first 12 weeks of gestation. Urorectal septum divides cloaca into dorsal rectum and ventral urogenital sinus. Trigone develops from dilation, fusion and incorporation of caudal mesonephric ducts into urogenital sinus, forming a triangular area. Posterior walls, dome and part of lateral walls arise from mesenchyme surrounding urogenital sinus. Anterior wall and part of lateral walls develop with closure of infraumbilical portion of abdominal wall.

Normal histology

Bladder layers are mucosa (urothelium, lamina propria, discontinuous muscularis mucosa), muscularis propria, adventitia, serosa.

Urothelium: formerly called transitional epithelium since intermediate between nonkeratinizing squamous and pseudostratified columnar epithelium; 5-7 cell layers thick in contracted bladder, 2-3 cells thick in distended bladder; lines renal pelvis, ureters, bladder, most of urethra but not terminal urethra.

Superficial urothelium is single layer of umbrella cells, which are large and elliptical with abundant eosinophilic cytoplasm and often binucleation or

prominent nucleoli; one umbrella cell covers several underlying cells; inconspicuous in distended bladder; contains trilaminar (asymmetric) unit membrane composed of two dense layers of unequal thickness and a central lucent layer, and apical plaques containing uroplakins.

Intermediate urothelial cells are cuboidal to low columnar cells with well defined borders, amphophilic cytoplasm rich in glycogen; nuclei are regularly arranged, ovoid with long axis at right angles to surface; chromatin is finely granular; small nucleoli; usually no mitotic figures.

Basal urothelial layer is in contact with the basement membrane and it is typically only one cell layer thick. The basal cells are small cuboidal cells with small nuclei but higher nuclear / cytoplasmic ratios than the overlying superficial and intermediate cells. [19]

Lamina propria: contains loose to dense connective tissue, thin-walled blood vessels that may be close to epithelium, lymphatics, variable adipose tissue; also discontinuous muscular mucosa. Only 5% of bladders have well developed muscularis mucosa.

Muscularis propria: consists of inner longitudinal, circular and outer longitudinal layers of thick muscle bundles (layers are distinct only near bladder neck), may also contain adipose tissue between muscle fascicles, paraganglia.

Epithelial variants in the lower urinary tract

Because of its diverse embryonal origin, the lower urinary tract may be partially lined by epithelia other than the urothelium. These are

- Squamous epithelium of vaginal type
- Intestinal type of glandular epithelium
- Brunn's nests and cystitis cystica.

Squamous epithelium of the vaginal type

The trigone of the bladder in approximately 50% of normal adult women and in a small proportion of men contains area of non keratinizing squamous epithelium of the vaginal type. In cystoscopy, these area may appear as a gray membrane.

Intestinal – type epithelium

Because the embryonal intestinal tract (the cloaca) participates in the formation of the lower urinary tract, areas of mucus producing intestinal type epithelium with goblet cells may occur in the bladder. In most patients, these areas are small, but occasionally the bladder may be fully or partially lined by this type of epithelium with a high risk to develop adenocarcinoma.

Brunn's nests and cystitis cystica

The urothelium of the bladder may form small, usually round buds, known as the nests of Von Brunn(brunn's nests) that extend into the lamina propria, occasionally to the level of the muscularis. They occur in approximately 80% of normal bladder. Within the centre of Brunn's nests, there is often formation of cysts which may be lined by mucus producing columnar epithelium. The cysts may become quite large and distended with mucus giving rise to, so called cystitis cystica or glandularis.

Methods of specimen collection

1) Voided urine
2) Catheterized urine
3) Bladder washings or barbotage

1) Voided urine

Voided urine is the simplest method of collection. Although an early morning urine specimen is the most useful one for other pathological investigations such as microbiology it is next to useless for cytological evaluation. Exfoliated cells lying in urine for several hours are usually too degenerate for accurate evaluation. It is recommended that a mid morning or random specimen is sent to the laboratory quickly for processing. If a short

delay is inevitable the container may be placed in a refrigerator; with an unavoidable longer delay, equal volume of 50% alcohol should be added to the sample to fix the cells. The sensitivity of a single voided specimen for bladder neoplasia was 97% in one report rising to 100%, when two or more specimens were examined. However ideally at least 3 urine specimens should be examined.[47]

Advantages

- Non invasive
- Inexpensive
- Easily repeated

Disadvantages

- Low cellularity
- Prominent degenerative changes

2) Catheterised urine

These are collected only when clinically indicated as catheterization is an invasive procedure which can produce much discomfort.

Advantages

- Greater cellularity than voided urine

Disadvantages

- Increased risk of infection

- Instrumentation artifact
- Degenerative changes similar to voided urine
- High cellularity and papillary like groups can lead to over diagnosis of urothelial carcinoma

3) Bladder washings

Indicated for work up of suspected or recurrent bladder cancer. Bladder washings with normal saline or Ringer's solution performed at cystoscopy, if indicated in combination with biopsies or resections.

It is the specimen that should be used for flow cytometric studies. The washings should be collected in equal amounts of alcohol for fixation.

Advantages

- Excellent preservation
- High cellularity
- High sensitivity, including low grade tumours
- Less contamination

Disadvantages

- Inconvenient, uncomfortable expensive
- Possible risk of infection spread of tumours.

PREPARATION TECHNIQUES

Smears of fresh and fixed specimens

Direct smears may be prepared after centrifugation of 50 ml of urine for 10 minutes at 1200 rpm. Albuminized slides or the addition of celloidin solution are recommended for better attachment of cells to the slides. The smears are then stained with papanicolaou stain. The papanicolaou stain is usually preferred, since fine nuclear detail is often crucial to proper diagnosis. Romanowsky, toludine blue and sternheimer-Malben stains have also been used, often as an adjunct to routine urinalysis.[25]

A number of other methods of urinary sample preparation are available including filter preparation, the so called saccomano blending technique, routine cytocentrifugation (eg. Cytospin™) and liquid based technologies (eg surepath™ and Thin – prep™). Urine rarely contains adequate materials for the preparation of cell blocks, but if visible sediment or tissue fragments are present, a cell block should be prepared.

Cytological findings in normal urine

- Scanty cellularity in voided samples
- Umbrella cells, deeper layer cells, squamous cells seen
- A few polymorphs usually present
- Spermatozoa and corpora amylacea may be present in males.

DISEASES AND CONDITIONS ASSOCIATED WITH URINARY BLADDER

1) Stones (Lithiasis)

Highman (1982) emphasized that stones can cause an increase in cellularity even in voided urine specimens, including mechanical avulsion of pseudopapillary group of transitional epithelium with smooth or irregular borders mimicking papillary urothelial carcinomas. The nuclei may be enlarged and pleomorphic, irregular in size and shape, with an increased N/C ratios. The chromatin can be coarse and variably hyperchromatic. Prominent nucleoli as well as mitotic figures can be seen. Inflammation, blood and necrosis may be present in the background, mimicking a tumour diathesis. Columnar and multinucleated transitional cells are common. Squamous and occasionally glandular metaplasia occur in long standing cases.[8] Reactive changes closely mimic malignancy.

2) Inflammation (Cystitis)

Cystitis is a common condition at all ages although the underlying causes vary in different age group. The inflammatory process may be acute or chronic and is usually bacterial in origin, although viruses, fungi and parasites are also well recognized causes, Escherichia coli are the most frequent pathogens.

Predisposing factors for cystitis are diabetes mellitus, prostatism and immunosuppression. Non infective causes include neoplasm, lithiasis, radiation effects and chemical irritation.

Cytology findings

- Hazy or turbid urine specimen
- Numerous polymorphs, histiocytes, occasionally eosinophils
- Inflammatory changes in epithelial cells
- Organisms may be present
- Evidence of associated pathology may be seen.

Histopathology

The histopathologic changes may include ulceration of the epithelium and infiltration of the wall of the organ by granulocytes in the acute phase and lymphocytes in the chronic phase.

Interstitial (Hunner's) cystitis

It is of unknown etiology, primarily affects middle age women and may be debilitating in some cases. The lesion can be located anywhere in the bladder.

Cytology

There may be marked but nonspecific increase in the number of reactive transitional cells. An increase in number of mast cells reported in bladder washings,[18] but their diagnostic significance is disputed.[23]

Histopathology

There is mucosal ulceration covered by fibrin and necrotic material. The underlying lamina propria and muscularis show edema, hemorrhage, granulation tissue and a mononuclear inflammatory infiltrate. Mast cells are usually present beneath the ulcer.

Eosinophilic cystitis

Most commonly observed after cautery treatment and may also occur in patients with asthma or other allergic disorders. Spontaneous forms of eosinophilic cystitis may also occur. The cystoscopic appearance is that of diffusely edematous and erythematous mucosa with broad based polypoid growths that may simulate neoplastic process.

Cytology

Urinary sediment may contain numerous bilobed eosinophils.

Histopathology

A dense inflammatory infiltrate rich in eosinophils often accompanied by fibrosis and muscle necrosis and sometimes by giant cells is present.[4]

SPECIFIC INFECTIONS

Tuberculosis

Tuberculosis of the bladder is usually secondary to tuberculosis of the kidney.

Cytology

Multinucleated giant cell histiocytes, often with peripheral nuclei (Langhans' cells), may be identified.[48]Clusters of epithelioid histiocytes that often have a spindle or carrot shape may be found.[32]The nuclei are round to oval with fine chromatin and the cytoplasm is finely vacuolated with indistinct border. Acid fast bacilli or positive urine cultures confirm the diagnosis. Mild to severe reactive epithelial atypia may also be associated with the infection, which must be differentiated from malignancy.

Histopathology

The bladder wall shows presence of numerous epithelioid granulomas and Langhans' giant cells. Similar findings can also be seen in patients treated with Bacillus calmette Guerin for urothelial carcinoma or after bladder surgery.[53]

Schistosomiasis

Usually caused by schistosoma hematobium (occasionally schistosoma mansoni), rare in this country, is extremely widespread in certain parts of

Africa, particularly along the Nile river. It is associated with squamous cell carcinoma of the bladder.

Cytology

Numerous anucleated squames and squamous cells corresponding to squamous metaplasia may be seen. Schistosomal ova with a terminal spine can sometimes be found in the urine.[28]

Histopathology

Schistosoma ova provided with terminal spine are deposited mainly in the submucosa of the bladder. The ova cause severe inflammatory reaction and fibrosis of the bladder.

Viral Infections

Viral infections such as herpes, cytomegalovirus, Human polyoma viruses etc. can occur in the bladder urothelium. Cytopathic changes induced by human polyoma virus may be mistaken for malignancy.

Fungal Infections

Fungal infections of the bladder can be an isolated finding or detected as part of systemic fungal disease.They are more commonly seen in immunosuppresed or diabetic patients.Most common types are Blastomyces, Cryptococcus, Aspergillus and Candida.[46] A polymorphonuclear leucocytic response may be seen in urine.

Malacoplakia

Bladder is the most common site. More common in immunocompromised. Also more common in women. Caused by defects in phagocytic or degradative functions of histiocytes in response to gram negative coliforms(E coli, proteus).

Cytology

Some histiocytes containing characteristic Michaelis-Gutmann bodies can be seen in voided urine. These bodies are PAS – positive and also stain for iron and calcium.

Histopathology

Foamy epithelioid histiocytes with PAS(+) granular eosinophilic cytoplasm seen in lamina propria. Some lymphocytes and occasional giant cells are seen. Histiocytes have increased number of phagosomes containing non digested bacteria (Michaelis- Gutmann bodies).

Neoplasms of the urinary bladder

Neoplasms of the bladder pose biologic and clinical challenges. The incidence of bladder epithelial tumours in the United States has been steadily increasing during he past years and is now more than 57,000 new cases annually.[2]

About 95% of bladder tumours are of epithelial origin, the remainder being mesenchymal tumours. Most epithelial tumours are composed of urothelial (transitional) type cells and are thus interchangeably called urothelial or transitional tumours, but squamous and glandular carcinomas also occur. Urothelial carcinoma (UC) is two to three times more common in men than women.

THE WHO / ISUP CONSENSUS CLASSIFICATION

. The consensus classification of the World Health Organization / International Society of Urologic Pathology (WHO/ISUP) was published in 1998.[17] The WHO/ISUP classification recognizes the existence of five general diagnostic categories for lower urinary tract biopsies:

1. Normal
2. Hyperplastic lesions
3. Flat lesions with atypia
4. Papillary neoplasms
5. Invasive neoplasms

WHO/ISUP (1998) classification of urothelial neoplasms

Classification:

- Normal
  - Normal
- Hyperplasia:
  - Flat hyperplasia,
  - Papillary hyperplasia
- Flat lesions with atypia:
  - Reactive (inflammatory) atypia,
  - Atypia of unknown significance,
  - Dysplasia (LG IUN),
  - CIS (HG IUN)
- Papillary urothelial neoplasms:
  - Papilloma,
  - Inverted papilloma,
  - Papillary neoplasm of low malignant potential,
  - Noninvasive papillary carcinoma-low grade,
  - Noninvasive papillary carcinoma-high grade
- Invasive urothelial neoplasms:
  - Lamina propria invasion,
  - Muscularis propria (detrusor muscle) invasion

The WHO/ISUP classification scheme is a histologic classification system for biopsy specimens and therefore utilizes both cytologic and architectural criteria for subclassification of urothelial lesions. For this reason, many of the diagnostic entities in the WHO/ISUP system cannot be diagnosed in urinary tract cytology specimens. For example, ‘flat hyperplasia’, papilloma, and even papillary neoplasm of low malignant potential are difficult if not impossible to diagnose in cytology specimens because, their cytologic features are very similar to those of normal urothelium. On the other hand, most cases classified by the WHO/ISUP scheme as low and high grade urothelial carcinoma (UC) will yield diagnostic cells in urinary cytology specimens. These, of course, are the most clinically important lesions, and the WHO/ISUP committee recognizes the importance of urinary cytology for screening and monitoring patients for urothelial carcinoma. Whenever possible, the cytologic diagnosis should be related to the corresponding diagnostic term in the WHO/ISUP system in order to foster uniform terminology and improve communication between pathologists and urologists. To the best of our knowledge, no publication has been reported utilizing this classification scheme in the evaluation of cytologic specimens.

Epidemiology

In the United States, tumors of the bladder are the fourth leading type of cancer in men but are less common in women (Messing and Catalona. 1998).

It is estimated that 33% of bladder cancer cases are related to tobacco smoke.[29] Cigarrete smokers have a 2-3 fold increase in risk of bladder cancer compared to non smokers.[39]

For the year 2001, the American Cancer Society projected more than 54,000 new cases and 12,400 deaths from tumors of the bladder .[21]

The impact of environmental factors on the genesis of tumors of the bladder has been known since the publications by the German surgeon Rehn (1895 and 1896), who observed that workers in factories producing aniline dyes were at a high risk for this disease.

It was subsequently shown that the carcinogenic compounds to which these workers were exposed were aromatic amines, such as 2-naphthylamine, para-aminodiphenyl (xenylamine), and 4-4-diaminobiphenyl (benzidine) Bonser et al, 1952; Boyland et al, 1954). Another compound known as MBOCA [4, 4' methylenobis (2-chloroaniline)], an analogue of benzidine, has been shown to induce low-grade papillary tumors in the bladder .[57]

The drug chlornaphazine, related to the aromatic compounds, was shown to be carcinogenic for the bladder .[35]

Women working in factories producing phenacetin, a common analgesic, and heavy users of the drug are also at increased risk for urothelial tumors that may involve not only the bladder, but also the ureters and the renal pelves .[30]

There also is evidence that workers in rubber and cable, leather, and shoe repair industries are at a high risk for bladder cancer, although the specific carcinogenic substances have not been clearly identified.

Norrier at al (2000) reported that the use of a Chinese herb (aristolochia fangchi) may also be a risk for bladder tumors.

A high level of inorganic arsenic in drinking water is another cause of bladder cancer.[15]

Besides the environmental factors, there are other risk factors for tumors of the bladder. For example, paraplegic and quadriplegic patients are at risk, presumably because of inadequate voiding, and therefore exposure of the bladder to small doses of unknown carcinogenic agents contained in the urine.[33]

Similar mechanisms may be responsible for bladder tumors in otherwise normal men with low intake of fluids (Michaud et al, 1999) and enlargement of the prostate.

Barlebo and Sorensen (1972) observed 2 patients with carcinoma in situ of the bladder, initially seen because of prostatic hypertrophy. A further association of bladder cancer with prostatic disease was reported by Mahadevia et al (1986). Nickel et al (2002) reported that three urothelial carcinoma in situ

were observed among 150 patients with chronic prostatitis evaluated by urine cytology.

Tumors of the bladder are observed with high frequency in some geographic areas. In the United States, these tumors are often observed in the state of New Jersey and in New Orleans, presumably because of a high level of exposure to industrial waste. Egypt and many other African countries, an infection with the parasite Schistosoma haematobium (Bilharzia) is an important cause of bladder cancer. It is speculated that industrial pollution, cigarette smoking or a combination of these and other yet unknown factors contribute to cancers of the lower urinary tract.

The term urothelial tumors has now been accepted by consensus of urologic pathologists (Epstein et al, 1998).

The urothelial tumors of the bladder may be classified into two fundamental, although to some extent overlapping, groups with different patterns of behavior, different prognoses and different cytologic presentation. These are:

- Papillary tumors
- Nonpapillary tumors

The papillary tumors of the urothelium have for the most part, a different natural history from the nonpapillary, flat tumors. It is of particular importance to recognize that many common, well-differentiated papillary tumors (low-

grade tumors) should not be classified as “carcinomas” because they do not, or very rarely, progress to invasive cancer. On the other hand, non-papillary or flat urothelial lesions (carcinoma in situ and related abnormalities) are the principal precursor lesions of invasive urothelial cancer.

Current theory suggests that UC may occur as one of the two distinct disease processes, each of which has a distinct cytological appearance and a distinct clinical course: low grade UC and high grade UC.[58]

The majority of UC are low grade tumours; they have a papillary growth pattern and infrequently invade and metastasize. As a consequence, they are biologically indolent and are associated with long patients survivals. Cytologically, most papillary UCs are low-grade and may be difficult to distinguish from normal or reactive urothelial cells in voided urine specimens or bladder washings. High- grade UCs exhibit a sessile or nodular growth pattern with a high likelihood of invasion and metastasis. Not surprisingly, these tumours are associated with poor patient survival. The cytology of these tumours is almost always high-grade. [41]

Histological Grading of Papillary Tumors

In 1922, Broders, of the Mayo Clinic, observed that the behavior of papillary tumors of the bladder depended significantly on the morphologic make-up of their epithelium and introduced the concept of tumor grading.

Classification and Grading of Papillary Tumors of the Bladder

	Number of epithelial cell layers	**Superficial cells**	**Nuclear enlargement**	**Abnormalities hyperchromasia**
Papilloma	No more than 7	Present, albeit small	Not significant	Absent
Papillary tumors grade I (papillary neoplasm of low malignant potential)	More than 7	Usually present, albeit small	Slight to moderate	Slight in occasional cell
Papillary tumors grade II (papillary carcinoma, low grade)	More than 7, usually marked increase	Variable	Moderate to marked	Slight to moderate in 25-50% of cells
Papillary carcinoma grade III	More than 7, often marked increase	Usually absent	Marked; extreme variability of sizes	Marked in 50% or more of cells

Histological grading of Invasive carcinomas of the bladder

Invasive carcinomas of the bladder composed of orderly sheets of cells resembling normal urothelium (grade I tumours) are very rare. Virtually all invasive tumours are grade II, III, or IV, depending on the level of architectural and cytologic abnormality. Grade II tumours mimic papillary tumours of high grades and are composed of sheets of relatively uniform cancer cells separated from each other by bands of connective tissue. Grade III tumours are usually solid and are characterized by variability in the size of cancer cells and marked nuclear abnormalities. Grade IV tumours are either composed of large cancer cells, spindle and giant cells, or of small cancer cells. [37]

RECOGNITION OF SPECIFIC TYPES OF UROTHELIAL TUMORS IN URINARY SEDIMENT

Papillary Tumors of Low Grade (Papillomas and Grade I Papillary Tumors of Low Malignant Potential)

In the presence of these tumors, the background of the cytologic preparations is usually clean and there is rarely any evidence of inflammation or necrosis. Erythrocytes in varying numbers are usually present.

It has been suggested that there are some differences in the configuration of cell clusters between low-grade papillary tumors and normal urothelium.[31] It is true that the surface of the clusters of normal urothelium is often composed of

semilunar umbrella cells with smooth surface. However, clusters with “ragged borders” may also occur in a variety of benign conditions, such as instrumentation, inflammation, or stones (lithiasis). The latter condition, named “calculus artifact,” was discussed at length in a study by Kannan and Gupta (1999), who documented the presence of cell clusters with irregular borders and slight level of nuclear atypia in 46 of 65 patients with lithiasis.

Murphy et al (1984), who claimed that low-grade papillary tumors could be identified in 62% of patients.

Direct washing or brushings of the urinary bladder contribute little to the diagnosis of low-grade papillary tumors. Harris et al (1971) were able to diagnose such lesions only in cellblocks of the urinary sediment, wherein biopsy-sized fragments of such tumors were observed.

Raab et al (1994), using logistic regression analysis of numerous parameters, suggested that irregular nuclear borders, increased nucleo/cytoplasmic ratio, and cytoplasmic homogeneity of urothelial cells in bladder washings were highly specific for low-grade tumors.

The same group of investigators confirmed that the three criteria were valid in Thin Prep preparations with a sensitivity of 59% and specificity of 100% (Xin et al, 2003).

Renshaw et al (1996) failed to confirm these observations.[49] Bastacky et al (1999) also were unable to recognize cell features characteristic of low-grade

lesions.[7] Sack et al (1995), in a cohort of 208 patients, recognized low grade papillary tumors in 11 of 33 such patients but also committed an equal number of false-positive errors.[50] Thus, cytology of the urinary sediment does not lend itself to the diagnosis of papillary tumors of low grade.

Papillary Tumors Grade II (Papillary Carcinoma, Low Grade)

Not all grade II tumors can be recognized cytologically. In 20 of 68 such tumors studied by Koss et al (1985), only benign or somewhat atypical urothelial cells were observed, and the diagnosis could not be established. In aneuploid papillary tumors, grade II, markedly typical or frankly malignant cells can be recognized, either singly or in small clusters. The cancer cells are usually of medium size and rarely show marked abnormalities of configuration, as in tumors of higher grade.

Papillary Tumors Grade III (Papillary Carcinomas, High Grade)

All or nearly all papillary tumors grade III can be identified by cytology. These tumors shed cancer cells that are of variable size and configuration. The papillary tumors, even highly anaplastic, may shed cancer cells in large clusters, sometimes reminiscent of papillary arrangement of cells. However, single cancer cells are always present and are usually numerous. The background of smears often, but not always, shows evidence of inflammation and necrosis.

Nonpapillary Urothelial Carcinoma

These lesions, particularly the nonpapillary carcinoma in situ and related lesions (IUN of high grade), are the principal target of cytologic studies of the urinary tract.

Nonpapillary (Flat) Carcinoma in Situ

Voided urine sediment is the ideal diagnostic medium for the primary diagnosis of nonpapillary carcinoma in situ, whether located in the bladder, the renal pelvis, the ureters, or the urethra. Regardless of the method of preparation, the urinary sediment usually yields persuasive evidence of cancer, reflecting the poor adhesiveness of cancer cells in the epithelial lesion.

The most common cytologic presentation of flat carcinomas in situ is a fairly monotonous population of medium-sized or small urothelial cancer cells, comparable in size to benign urothelial cells from deeper layers of the urothelium. The cancer cells usually appear singly, but occasionally form small clusters. Occasionally, a few larger or bizarre cells may occur. Regardless of size, the cells have an irregular configuration and relatively scanty, usually basophilic cytoplasm, although cells with eosinophilic cytoplasms may occur. The nuclei are relatively large, hyperchromatic, have an irregular contour and show an abnormal chromatin texture. A coarse, filamentous arrangement of the chromatin is especially frequent. Enlarged nucleoli are infrequent but may

occasionally be noted. Condensation of nuclear chromatin (pyknosis) is fairly common and, in such nuclei, the arrangement of chromatin cannot be studied.

In the presence of carcinoma in situ, the urine rarely contains more than a few inflammatory cells or erythrocytes, and there is usually little evidence of necrosis, whereas marked inflammation and necrosis are commonly observed in invasive cancer.

Invasive Nonpapillary Urothelial Carcinoma

In the cytologic preparation, there is usually evidence of marked inflammation, bleeding, and necrosis. In fully developed cancer, the predominant cancer cells are of variable sizes, of irregular configuration, with scanty cytoplasm and prominent, obviously abnormal, hyperchromatic nuclei, similar to cancer cells observed in high-grade papillary tumors. Although most cancer cells have a basophilic cytoplasm, the presence of single keratinized cancer cells with eosinophilic cytoplasm is not rare. Sometimes, early invasive carcinoma may give a smear pattern identical with carcinoma in situ. In some advanced cancers with necrotic surface, the yield of cancer cells may be very low.

HISTOLOGIC VARIANTS OF UROTHELIAL CARCINOMA

Squamous (Keratinizing) Carcinoma

The presence of a focal squamous component in urothelial carcinoma is a common finding. Rarely, low-grade papillary tumors may have a squamous components. Also condylomata acuminata may be mistaken for squamous carcinoma in situ.

Bladder cancers made up predominantly or exclusively of squamous (keratinizing) cell types are less frequent in the Western world than urothelial carcinoma although they are common among patients with Schistosoma hematobium infestation. It is generally assumed that such tumors originate from areas of squamous metaplasia or leukoplakia, although this cannot always be conclusively documented. Squamous carcinomas, like urothelial carcinoma, may be graded according to the degree of differentiation (Koss, 1975).

Cytology

In most cases, the cytologic presentation of squamous carcinoma of the urothelium closely resembles similar lesions of the uterine cervix and bronchus. The tumors shed squamous cancer cells, some of bizarre configuration, with eosinophilic, often markedly keratinized cytoplasm. The nuclei are pyknotic and occasionally may be totally submerged by keratin formation, with resulting

formation of “ghost” cells, not unlike those observed in squamous carcinoma of the lung. Clusters of cancer cells are common in bladder washings.

In the very rare squamous papillary urothelial tumors of low grade, concentrically arranged squamous cells or “squamous pearls” may occasionally appear in urinary sediment. Condylomata acuminata of the bladder may mimic the cytologic finding in squamous carcinoma.

In a study performed at Memorial Hospital in New York City on urine sediments mailed in plastic bags from Bulawayo, Zimbabwe, the cytologic diagnosis of cancer could be rendered in only 15 and 29 patients with schistosomiasis and proved cancer of the bladder.[28] Similar observations were made by Dimette (1955) and El-Bolkainy and Chu (1981).

Adenocarcinoma

Occasional foci of glandular differentiation in urothelial carcinoma are common. These focal changes cannot be recognized in cytologic samples. Primary adenocarcinomas may occur anywhere in the lower urinary tract, most commonly in the bladder, but occasionally in the renal pelvis or the ureter. Risk factors for adenocarcinoma of the lower urinary tract are: extensive intestinal metaplasia, extrophic bladders and the benign villous adenoma, a polypoid lesion lined by intestinal epithelium, similar to lesions observed in the colon.[22] Such tumors may also arise in cystitis glandularis and nephrogenic adenomas.

Adenocarcinomas of the urothelium are predominantly of enteric type. Most of such tumors closely resemble carcinomas of the colon and may be made up of columnar, mucus-producing cells or signet-ring type cancer cells. Adenocarcinoma in situ of the bladder has been observed. The rarity of primary, uncomplicated adenocarcinoma in situ was recently emphasized by Chan and Epstein (2001).[12]

Nazeer et al (1996) described an adenocarcinoma in situ of endocervical type developing in a case of a woman harboring endocervical type glands in the wall of the bladder.

Bladder adenocarcinomas of clear cell type, resembling vaginal lesions occurring in daughters of Diethyl stilbestrol-exposed women, may occasionally be observed.[44] Amin et al (1994) described an exceedingly uncommon type of adenocarcinoma, resembling ovarian serous carcinoma, and named it micropapillary variant of urothelial carcinoma.

Adenocarcinomas derived from the urachus (remnants of the embryonal omphaloenteric duct) arise in the dome of the bladder and along the course of the urachus, terminating at the umbilicus. A patient reported by Hom et al (1990) had an adenocarcinoma with endocrine component.[27]

Cytology

In fortuitous cases, adenocarcinomas can be recognized in the urinary sediment because they shed cells resembling those of colonic carcinoma. These

are often columnar in configuration and have large, hyperchromatic nuclei and vacuolated cytoplasm. Such cells may form clusters that may show a spherical or rosette-like arrangement. Numerous elongated or columnar cancer cells have been observed in the smear of the urinary sediment.

Occasionally, somewhat smaller and more spherical cancer cells, with large, peripheral nuclei and vacuolated, mucin-containing cytoplasm, resembling signet ring type cells of intestinal cancer, may be observed. The presence of very small signet ring cells in a woman may also indicate a metastatic mammary lobular carcinoma. In many cases, however, the sediment contains undifferentiated cancer cells and adenocarcinoma cannot be identified.

Bardales et al (1998) reported a patient with urachal adenocarcinoma whose urinary sediment showed bland columnar cells and mucin.[6]

In the rare cases of clear-cell-type adenocarcinoma, papillary clusters of malignant cells with large nuclei, prominent nucleoli, and clear cytoplasm may be observed. Similar cases were reported by Peven and Hidvegi (1985) and Doria et al (1996).[16]

UNCOMMON TUMOROUS CONDITIONS AND TUMORS OF THE BLADDER

Papillomatosis of Bladder

This is a rare disorder in which the entire surface of the bladder is covered with innumerable papillary fronds lined by essentially normal or minimally atypical urothelium. Little is known about the natural history of untreated papillomatosis (Koss, 1975). The lesion cannot be recognized cytologically.

Nephrogenic Adenoma (Adenosis of Bladder)

This uncommon lesion is composed of ducts and tubules, possibly of enteric origin (Koss, 1985, 1995). Stilmant et al (1986) reported the presence of markedly abnormal cells in four patients.[54] Three of the patients, however, had documented bladder cancer with carcinoma in situ. Troster et al (1986) observed papillary urothelial clusters in the urine of one patient.[56] The clusters had no specific features. It is doubtful that nephrogenic adenoma can be recognized in urinary sediment. However, adenocarcinomas may develop in such lesions and may shed cancer cells.

Endometriosis

Endometriosis of the lower urinary tract, particularly of the bladder, is a rate condition in young women that may cause symptoms similar to those

caused by a tumor. Schneider et al (1980) reported clusters of endometrial cells in voided urine in a case of endometriosis of the bladder.[52] There were some similarities between cells from endometriosis and metastatic endometrial carcinoma. Bohlmeyer and Schroyer (1996), in reporting another case, pointed out that the endometrial cells in clusters in voided urine may be mistaken for cells of a urothelial carcinoma.

Eosinophilic Granuloma

The rare eosinophilic granuloma may occur in the bladder or ureter. Because the lesion is subepithelial, there are no known specific cytologic findings.

Amyloidosis

Large deposits of amyloid in the wall of the bladder may elicit a granulomatous reaction with foreign body giant cells mimicking a tumor (Koss, 1975). There is no record of this diagnosis in either urine sediment or in direct aspirates of bladder wall.

BENIGN TUMORS

Condylomata Acuminata

Condylomata acuminata may occasionally be observed in the urinary bladder (Koss, 1975).

Histology

The tumors, composed of folds of squamous epithelium, resemble genital condylomata acuminata, characterized by the presence of koilocytes in the superficial epithelial layers. In some tumors, marked nuclear abnormalities may be observed in epithelial cells.

Cytology

In voided urine, Koilocyte-like cells can be observed. Hartveit et al (1992) reported the presence of koilocytes in the urinary sediment of numerous patients with a variety of bladder lesions.[24]

More importantly perhaps, Koss et al, in two of the three patients, found the urinary sediment contained large, highly abnormal squamous cells with keratinized cytoplasm and large, hyperchromatic and pyknotic nuclei. Cell-in-cell arrangement was observed. Although in some of the cells perinuclear halos suggested a similarity to koilocytes, such cells were very difficult to distinguish from cells of squamous carcinoma.

Squamous Papilloma of Bladder

Cheng et al (2000) described five squamous papillomas of the bladder and two of the urethra.[13] The lesions failed to hybridize with HPV DNA and were diploid. These benign lesions are extremely rare and there is no information on their cytologic presentation.

Inverted Papilloma

An uncommon tumor of the urinary bladder, somewhat similar to a papilloma with a flat surface, was first described by Potts and Hirst in 1963. an uninterrupted layer of normal urothelium lines the surface of the tumor, which is made up of anastomosing strands of urothelium (Koss, 1975). The tumor is benign and there are no known cytologic abnormalities associated with it.

Villous adenoma

Villous adenoma is a rare tumor of bladder of enteric origin that resembles similar tumors of the colon and rectum (Koss, 1975). There are no known cell abnormalities in the urinary tract associated with this disorder.

TUMORS WITH MALIGNANT POTENTIAL

Pheochromocytoma (Paraganglioma)

Histology and Clinical Presentation

These endocrine tumors, classically composed of nests or cords of large, eosinophilic epithelial calls (Zellballen), separated from each other by thin mantles of richly Vascularized connective tissue, produce hormones, the catecholamines, that may cause episodes of paroxysmal hypertension on voiding. Most of these tumors are benign but malignant variants are known to occur (summary in Koss, 1975).

Cytology

Because these tumors are located within the wall of the bladder, there are no recorded cases of tumor cells in voided urine.

Carcinoids

Carcinoids of the bladder are morphologically identical to similar tumors occurring in the gastrointestinal tract and the lung and are composed of sheets and ribbons of small cells, sometimes forming glands. Although usually benign, these tumors may display malignant behavior, as in the example cited by Koss (1985). There is no record of cytologic findings in bladder carcinoids, but it may be assumed that the cytologic presentation would be similar to that of carcinoids in other organ.

Small-Cell (Oat Cell) Carcinomas

Small-cell carcinomas are either pure, composed of sheets and ribbons of small malignant cells akin to the oat cell carcinoma of the lung, or mixed, with either solid urothelial carcinomas or adenocarcinomas. In some of these tumors, a urothelial carcinoma in situ can be documented, strongly suggesting that the tumors are variants of urothelial cancer (Koss, 1975).

Cytology

Although small-cell carcinomas are uncommon, their cytology has been repeatedly described in recent years.[1] The tumor cells are small, about two or three times larger than normal lymphocytes, are usually of approximately equal

sizes and appear singly or in small chains and clusters, wherein molding of adjacent cells can be observed. The cytoplasm is very scanty, often not visible. The relatively large nuclei show fine granularity. Nucleoli are absent or very small. In some cases, larger cancer cells corresponding to urothelial carcinoma can be present next to the small cancer cells.

Spindle and Giant Cell Carcinomas

These tumors are uncommon and there are few reported cases in the literature .[26] We observed one such tumor in the wall of a bladder diverticulum. Although the diagnosis of a malignant tumor can be easily established in the urinary sediment, the exact identification of such exceedingly rare tumors is rarely, if ever, possible. A case of carcinosarcoma, arising in a bladder deiverticulum, was reported by Omeroglu et al (2002).[45]

Mesodermal Mixed Tumors (Heterologous Carcinosarcomas)

These exceedingly rare variants of urothelial carcinomas of the urinary bladder closely resemble similar tumors observed in the female genital tract. In a case observed by Koss et al, the urinary sediment contained a mixture of malignant cells, some of which had features of urothelial carcinoma and others had features suggesting a chondrosarcoma.

Lymphoepithelioma-Like Carcinoma

These exceedingly uncommon tumors of the bladder are similar to nasopharyngeal tumors.[3] There is no information on the cytologic presentation of these tumors.

Nested Variant of Urothelial Carcinoma

This is an uncommon type of urothelial cancer with insidious onset. The tumor is difficult to recognize in biopsies because of its deceptively benign presentation: it is composed of nests and gland-like formations of urothelial cells that are approximately cuboidal and have only moderately enlarged, hyperchromatic nuclei.[9] The nested carcinoma apparently is derived from surface epithelium that is only minimally atypical and does not show any evidence of carcinoma in situ or related lesions (Young and Oliva, 1966; recently confirmed by Dr.Victor Reuter, MSKCC by personal communication). It is therefore not surprising that cytology of these tumors is nondiagnostic. Cardillo et al (2003) examined 13 urine sediments from 7 patients and reported that in nearly all cases, the tumor cells could not be differentiated from normal urothelial cells.[11]

Sarcomas of the bladder

These are mainly rhabdomyosarcomas and leiomyosarcomas (Koss, 1975) that occasionally shed cancer cells in urine, Krumerman and Katatikaru

(1976) described a case of a rhabdomyosarcoma with intraepithelial spread in an adult. Generally, cells of sarcomas in the urinary sediment have malignant features, but usually cannot be accurately identified in the absence of prior histologic diagnosis or clinical history, including the age of the patient.

In children, embryonal rhabdomyosarcomas (botryoid sarcomas) of the vulva, vagina, prostate, and urinary bladder may occur. The small cancer cells have no distinguishing features and could not be further classified, except by comparison with histology. By contrast, spindly cancer cells of adult forms of Rhabdomyosarcoma may show cytoplasmic striations.

A case of primary angiosarcoma of the bladder was reported by Schindler et al (1999) in an aspirated sample.[51]

Primary Melanomas

Primary melanomas of the bladder are exceedingly rare. Khalbuss et al (2001) described such a case in an 82 year old woman and summarized prior literature. In a personally observed case, the sediment of voided urine contained rare malignant cells with large nuclei and prominent nucleoli, some containing brown melanin pigment in the cytoplasm. Phagocytized pigment was also observed in macrophages.

Choriocarcinoma

Sporadic cases or primary choriocarinoma of bladder in males have been reported (Weinberg, 1939). Such a case was reported from the laboratory.[43] The sediment of voided urine contained numerous malignant cells, most of which resembled cells of urothelial carcinoma but some that were large and multinucleated, with large nuclei and nucleoli, consistent with syncytiotrophoblasts.

MATERIAL AND METHODS

The present study has been carried out in the Department of Pathology, Madurai Medical College, Madurai, India, for a period of 2 years from (September 2006 – August 2008).

Although bladder washings were considered superior to voided urine in detecting bladder lesions, voided urine was selected in our study because it is simple, safe, non invasive and inexpensive method for detecting bladder lesions.

Voided urine samples were obtained from 160 cases.114 cases from urology department Govt. Rajaji Hospital, Madurai and 46 cases from Urology department, Meenakshi Mission Research Centre, Madurai. 3 random voided urine samples were collected from each patient on 3 consecutive days and fixed in equal volume of 50% alcohol. The urine cytology smears were made after centrifugation and the smears were fixed in 95% alcohol, Papanicolaou staining and Modified Hematoxylin and Eosin staining were done (Annexure – I). The smears were analyzed and the cellular details were evaluated under light microscopy. The results were entered in the working proforma (Annexure –II).

Histopathology

Out of 160 cases, bladder biopsy specimens were obtained for 84 cases. 38 specimens from urology department Govt. Rajaji Hospital, Madurai and 46 specimens from Urology department, Meenakshi Mission Research Centre, Madurai.

The histopathology specimens of bladder biopsy were fixed in 10% neutral buffered formalin. The tissues were processed, paraffin blocked, 5 microns thin sections were cut and stained by Hematoxylin and Eosin as described by Bancroft in theory and practice of Histological Techniques.[5]

The results of histopathological study of H&E stained sections were recorded.

The information collected regarding all the selected cases was recorded in a master chart.

Sensitivity, specificity, accuracy, positive predictive value and negative predictive values were calculated using the following formulae and taking HPE findings as the Gold standard.

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive + False negative}} \text{X } 100$$

$$\text{Specificity} = \frac{\text{True negative}}{\text{True negative + False positive}} \text{ X } 100$$

$$\text{Accuracy} = \frac{\text{True positive + True negative}}{\text{Total number of cases}} \text{ X } 100$$

$$\text{Positive Predictive value} = \frac{\text{True positive}}{\text{True positive + False positive}} \text{X } 100$$

$$\text{Negative Predictive value} = \frac{\text{True negative}}{\text{True negative + False negative}} \text{X } 100$$

Photomicrographs of the smears and sections were taken wherever needed.

OBSERVATION AND RESULTS

In the two year study (September 2006 – August 2008) Voided urine samples were obtained from 160 cases.114 cases from urology department Govt. Rajaji Hospital, Madurai and 46 cases from Urology department, Meenakshi Mission Research Centre, Madurai. These urine specimens after fixation with equal volume of 50% alcohol were centrifuged and sediments were smeared into the glass slides.

Out of 160 urine cytology smears studied, urinary smear reports were within normal limits in 44 (27.5%). Inflammatory changes, non specific cystitis were 53 (33.1%).

Reactive changes were noted in 16 cases (10%). Malignancy changes were seen in 47 cases (29.4%).

Out of 160 cases, bladder biopsy specimens were obtained for 84 cases. A correlative study between urine cytology and histopathology was done in these cases.

Sex incidence

Out of total 160 cases, 125 cases (78%) were males & 35 cases (22%) were females. There were significantly greater number of males in the present study (Table-1 and Diagram-1).

Age Incidence

Age group starting from 19 to 85 was included in the study. Age 11-20 years were 3 (1.9%) 21-30 were 7 (4.4%) 31-40 years were 26(16.2%) 41-50 years were 25 (15.6%) 51-60 years were 47(29.4%) 61-70 years were 41 (25.6%) 71- 80 years were 10(6.2%) and age 81-90 was 1 (0.6%) (Table-2 and Diagram-2).

Incidence of smokers

Among 160 cases, 91 cases were smokers and 69 cases were non smokers. 72.8% of the male patients were smokers and 27.2% of the male patients were non smokers. All the female patients (100%) were non smokers. There was significantly higher number of smokers in the male population (Table-3 and Diagram-3).

Clinical symptoms

Among 160 cases, 53 patients presented with hematuria. 69 patients presented with dysuria and 47 patients presented with frequency of micturition. So majority of patients presented with dysuria as shown in (Table-4 and Diagram-4).

Past History

Among 160 cases, H/O recent surgery in the bladder was present in 14 cases. H/O bladder stones was present in 27 cases. H/O bladder tumours was

present in 13 cases. H/O pelvic irradiation was present in 12 cases. H/O TB was noted in 1 case as shown in (Table-5 and Diagram-5).

Cytological evaluation of urine smear reports

During this study, 160 urine cytology smears were received. They were stained and evaluated under standard techniques (Table-6 and Diagram-6).

We have reported normal study in 44 cases (27.5%) in which smears had scanty cellularity, with few superficial transitional cells, deeper layer cells, squamous cells and a few neutrophils (Fig-1).

Inflammatory changes were seen in 53 smears (33.1%) in which smears showed numerous neutrophils, histiocytes, lymphocytes & inflammatory changes in epithelial cells (Fig.2).

Reactive cases were 16 (10%). In these, smears showed reactive urothelial cells with enlarged cell size & increased nuclear/ cytoplasmic ratio, pleomorphic with irregular size & shape. These reactive cells are seen in clusters or singly.

Malignancy was diagnosed in 47 cases (29.4%). Urothelial carcinomas (UC) were categorized as Low-grade UC and High grade UC. Among which 4 cases(2.5%) were of low grade urothelial carcinoma. In this, cells are arranged in loose papillary clusters. Individual cells are uniformly enlarged with homogenous cytoplasm, increased nuclear/cytoplasmic ratio, with irregular

nuclear contour, fine and irregular nuclear chromatin with small or absent nucleoli (Fig – 3).

41 cases (25.6%)were of high grade urothelial carcinoma in which cells were arranged in loose clusters as well as seen isolated with enlarged and pleomorphic cells with vacuolated cytoplasm, increased nuclear cytoplasmic ratio, variable nuclear size, markedly irregular nuclear contour, coarse irregular chromatin with variable nucleoli, seen in a dirty back ground (Fig – 4).

Squamous cell carcinoma was diagnosed in 1 case (0.6%)in which smears were highly cellular with large pleomorphic cells with hyperchromatic nuclei, increased nuclear cytoplasmic ratio with scant eosinophilic cytoplasm in an inflammatory back ground (Fig – 5).

Adenocarcinoma was diagnosed in 1 case (0.6%)in which tumour cells were columnar, seen in clusters and have large, vesicular nuclei and vacuolated cytoplasm (Fig – 6).

Histopathological diagnosis

Out of 160 cases, bladder biopsy specimens were obtained for 84 cases. Bladder biopsy was reported as normal in 7 cases (8.3%) in which the bladder wall was lined by normal urothelium as shown in (Table-7 and Diagram-7).

Inflammations such as non specific cystitis were seen in 17 cases (20.2%) in which there was ulceration of epithelium and infiltration of the wall

of the bladder by granulocytes in acute phase and lymphocytes in chronic phase.

Tuberculous cystitis were found in 3 cases (3.6%) in which, the bladder wall showed numerous epithelioid granulomas and Langhans' giant cells (Fig – 9).

Schistosomasis was diagnosed in 1 African patient (1.2%) whose bladder biopsy specimen showed numerous schistosoma haematobium ova with terminal spine in the submucosa with surrounding inflammatory reaction and fibrosis (Fig – 10).

Benign neoplasms were seen in 2 cases (2.4%). 1 case was Leiomyoma of the bladder in which the tumour was composed of spindle smooth muscle cells arranged in fasicles (Fig – 11).

1 case was Transitional cell papilloma in which the individual finger like papillae had a central core of loose fibrovascular tissue covered by transitional epithelial cells that are histologically identical to normal urothelium.

Malignant neoplasms were seen in 54 cases (64.3%) (Diagram – 8). Urothelial carcinoma (UC) was classified as Papillary and Non papillary / Invasive (Diagram – 9). Papillary urothelial carcinoma grade I (low grade UC),was reported in 6 cases in which the tumour was composed of connective tissue stalks covered by delicate branching fronds of well differentiated cells,

layered more thickly than normal mucosa , but with very little atypia and few mitoses (Fig – 12).

Invasive urothelial carcinoma grade II (High grade UC)was found in 33 cases in which the tumour was composed of sheets of relatively uniform cancer cells separated from each other by bands of connective tissue (Fig – 13).

Invasive urothelial carcinoma III (High grade UC) was found in 13cases in which the tumour was solid, composed of cancer cells with variability in the size of cancer cells and marked nuclear abnormalities (Fig – 14).

Squamous cell carcinoma (SCC)was found in one case (1.2%), in which the tumour was composed of poorly differentiated tumour cells with marked nuclear pleomorphism and only focal squamous differentiation (Fig – 15).

Adeno carcinoma was found in one case (1.2%), in which the tumour was composed of pleomorphic tumour cells arranged in glandular pattern (Fig– 16).

DISCUSSION

This study is aimed at finding out the efficacy of urine cytology smear in detecting malignant lesions of the bladder in patients coming with bladder symptoms and correlate the cytological findings with histopathological diagnosis.

Sex incidence

In our study, 125 cases (78%) were males and 35 cases (22%) were females. There were significantly greater number of males in the present study. Malignant lesions were seen in 49 males and 5 females in histopathology specimen. Urothelial carcinoma was seen in 47 males and 4 females in the ratio of 12:1.

Boring CC et al (1994) found that bladder cancer also has a distinctive gender distribution with a 2.7 fold higher incidence in men than women. This difference is reflected in a nearly double death rate in men[10].

Age Group

In our study the age group 11-20 were 3 (1.9%), 21-30 were 7 (4.4%), 31-40 were 26 (16.2%), 41-50 were 25 (15.6%), 51-60 were 47 (29.4%), 61-70 were 41 (25.6%), 71-80 were 10 (6.2%), 81-90 was 1 (0.6%) with higher age incidence noted in 5^{th} and 6^{th} decades.

So the bladder pathology is more common in 5th and 6th decades of life.

Bladder cancers are more common in 6th and 7th decade.

Median age at diagnosis of bladder cancer in our study was 60 years. Youngest patient affected was 22 years old, eldest patient was 85 years old.

Squires TS et al (1994) in their study found that most bladder cancers develop in later adulthood, with median age at diagnosis over 65 years of age.

Ray B et al (1973) described in their study that although uncommon, bladder cancer can develop in young patients and rarely in children. The vast majority of the later are low grade papillary lesions, but rare examples of aggressive epithelial tumours, have been described in the urinary bladder.

Smoking Habit

In our study 91 patients were smokers and 69 patients were non smokers.

All 91 patients were male patients. Among 54 bladder cancers detected 45 patients had history of smoking.

In our study, it is estimated that 83% of bladder cancer cases were related to smoking.

Howe GR et al (1980) estimated that 33% bladder cancer cases are related to tobacco smoke. Cigarette smokers have a 2 to 4 fold increase in risk of bladder cancer compared to nonsmokers.[14]

The increased risk is similar in men and women and in different parts of the world.

Nitrosamines and 2-naphthylamine are known bladder carcinogens that are present in cigarette smoke, but whether they contribute to the increased risk to tobacco smokers remains unknown.[38]

Bladder Symptoms

In our study hematuria was present in 53 cases and dysuria in 69 cases and frequency of micturition in 47 cases.

Among 54 bladder cancers detected 45 patients presented with hematuria. So most common symptom of bladder cancer is hematuria.

Past history

In our study 27 cases had history of bladder stones. 14 cases had history of recent surgery in the bladder. 13 cases had history of bladder tumor, 1 case had history of tuberculosis (TB) and 12 cases had history of irradiation.

In our study, there was only one calculus artifact, which mimicked high grade urothelial carcinoma (Fig – 7) and one case where there was history of TB, had also tuberculous cystitis in histopathology.

In 1982 Highman W and Rubben H et al postulated that stone disease is perhaps the most important source of false positive diagnoses because it induces cytologic change in urothelium that mimic high grade urothelial carcinoma.

In the present study, there was one radiation induced artifact that mimicked high grade urothelial carcinoma in which the urine cytology showed more cellularity and the cells were irregularly enlarged, with increased nuclear/cytoplasmic ratio, irregular hyperchromatic nuclei with karyorrhexis and cytoplasmic hyperchromasia (Fig – 8).

In 1991 Yazdi HM found that many chemotherapeutic and radiotherapeutic agents induce cytologic changes in urothelial cells that may mimic malignancy.

In 1997 Kern WH et al stated that procedures such as catheterization, cytoscopy and retrograde studies all served useful diagnostic functions, but they can also complicate the work up, because they can dislodge large fragments of urothelial cells and produce urinary tract specimens that are alarmingly cellular and contain papillary groups. They can be mistaken for low grade urothelial carcinoma. These cellular changes can persist for weeks after instrumentation.

Cytohistopathological correlation

In our study cytohistopatholgical correlation was done in 84 cases as shown in (Table-8).

20 cases were normal cytologically. Among these 20 cases, histopathology report in 1 case was normal, 7 cases were non specific cystitis, 1 case was tuberculous cystitis and 11 cases were urothelial carcinoma.

So not all cases of non specific cystitis, tuberculous cystitis and urothelial carcinomas are detected by urine cytology. False negativity may also be due error in screening or some of the urothelial carcinomas, such as low grade tumours rarely shed malignant cells.

Inflammatory changes were reported in cytology smears in 13 cases. Among these 13 cases, in histopathological correlation 1 was normal, 1 was tuberculous cystitis, 8 were cystitis, 3 were reported as urothelial carcinoma.

Reactive changes were reported in cytology in 10 cases. In histopathological correlation 4 were normal, 1 was tuberculous cystitis, 1 was schistosomiasis reported in 1 African patient, 2 were cystitis, 2 were benign neoplasms, 1 was leiomyoma and 1 was transitional cell papilloma.

Benign neoplasms, such as leiomyoma and transitional cell papilloma induce only reactive changes in the urothelial cells that are shed in urine. They do not cause any specific changes.

Kapila and Verma (1984) described the presence of comma-shaped epithelioid cells in the urinary sediment of a patient with tuberculosis of the bladder. The slender, carrot-shaped cells forming a tubercle are characteristic, if present. Piscioli et al (1985) described the cytologic findings in the urinary sediment of 11 patients with tuberculosis. In 5 of them, he reported finding epithelioid cells, although the illustration provided was not convincing. In all 11 patients, multinucleated cells of Langhans' type were observed. In Koss's

experience, this type of giant cells is extremely rare in urinary sediment and its presence has yet to be proven to be of diagnostic value. Piscioli et al also described in 2 patients the presence of markedly atypical urothelial cells resembling cancer cells, which they traced to atypical hyperplastic urothelium that was similar to flat carcinoma in situ.

In a study performed by Houston et al, in 1966 found that numerous anucleated squames and squamous cells corresponding to squamous metaplasia were observed in 18 of 51 urine sediments from patients from Zimbabwe with schistosomiasis. Ova were not seen in this material. Somewhat similar observation were reported by Dimmette et al (1955).

Because of air travel and movement of infected people, the finding of schistosoma haematobium is no longer confined to endemic areas. Clements and Oko (1983) reported such a case from New York City, and more such cases may be expected to occur in the Western world.

41 cases were reported as malignant smears in cytology. Among these 4 cases were diagnosed as Low-grade UC,35 cases as High grade UC,1 case as Squamous cell carcinoma and 1 as Adenocarcinoma.

In histopathological correlation, 4 cases diagnosed as Low-grade Urothelial carcinoma(UC) in cytology turned out to be Papillary urothelial carcinoma grade I (low grade UC)in histopathology, 35 cases diagnosed as High grade UC in cytology, turned out to be Invasive urothelial carcinoma

grade II (High grade UC)in 24 cases, Invasive urothelial carcinoma grade III (High grade UC)in 10 cases and one was reported as normal in bladder biopsy.1 case reported as squamous cell carcinoma (SCC) in urine cytology was also SCC in biopsy.1 case reported as Adenocarcinoma in cytology was also found to be Adenocarcinoma in biopsy.

One false positive case in cytology, which was diagnosed as high grade UC, but found to be normal in biopsy was due the bladder stones that had shed atypical urothelial cells that mimicked High-grade UC.

Coming to the efficacy of urine cytology in detecting bladder malignancy,

True positive cases were 40

False positive cases were 1

True negative cases were 29

False negative cases were 14

So the over all sensitivity was 74.1%, specificity was 96.7%. Accuracy was 82.1% positive predictive value was 97.6%, negative predictive value was 67.4% (Table -9 and Diagram-10)

A comparative study has been done with the findings of previous study groups (Table – 10).

The overall sensitivity in the detection of bladder cancer by different study groups was ranging from 50% to 97%. The present study indicates the sensitivity to be of 74.1%.

The sensitivity of Low-grade UC and High-grade UC in our study was 62.7% and 74% respectively. In Murphy WM et al (1984) the sensitivity of Low-grade UC and High-grade UC was 0-73% and 95% respectively.[40] (Table -11).

In both of these studies, the sensitivity of urine cytology in detecting High -grade UC was more than Low-grade UC indicating that High-grade UC shed malignant cells more readily.

Correlative study for proportion of positive malignant cases detected by cytology (Table -13)

In Kern W H study (1997) the proportion of positive malignant cases detected by cytology for Urothelial carcinoma grade I was 31%, grade II-44%, grade III-72%, for Squamous cell carcinoma-75% and Adenocarcinoma-63%.[34]

In our study, the proportion of positive malignant cases detected by cytology for Urothelial carcinoma grade I was 66.7%, grade II-72.7%, grade III-76.9%, for Squamous cell carcinoma-100%, Adenocarcinoma-100% (Table -12).

14 cases were found be false negative. Low grade urothelial tumors rarely shed cells in urine.

In 1990 Murphy WM postulated that only 30-60% of grade I urothelial carcinoma exfoliate carcinoma cells. The carcinoma cells in voided urine samples closely resemble normal urothelial cells.

There was 1 false positive case in our study. It may be due to inadequate biopsy or misinterpretation of benign process. False positivity may be due to bladder calculi or recent catheterization or recent surgery in the urinary tract which may dislodge cluster of urothelial cells which may mimic urothelial carcinoma.

SUMMARY

The present correlative study revealed the following;

1) The peak age incidence of Bladder pathology is seen in 5^{th} and 6^{th} decade. While bladder cancers are seen in 6^{th} and 7^{th} decades of life.
2) Bladder cancers are more common in male patients.
3) Smokers are more vulnerable to Bladder cancers.
4) Hematuria is the most common symptom in patients with bladder cancer.
5) The Most common malignant bladder lesion is urothelial carcinoma accounting for 96.3% of cancers in histopathology.
6) Among the urothelial carcinomas, Grade II Invasive urothelial carcinoma is more common.
7) Urine cytology is not good for diagnosing Noncancerous conditions.
8) The overall sensitivity of urine cytology is 74.1% specificity is 96.7% and accuracy is 82.1%.
9) The sensitivity of Low-grade UC and High-grade UC is 62.7% and 74% respectively.
10) Histopathological examination of the bladder is the gold standard against which the accuracy of urine cytology smears can be obtained and is found to 82.1% in this study.

CONCLUSION

In summary, a correlative study of urine cytology and histopathological examination of bladder lesions revealed the overall sensitivity of 74.1%, specificity of 96.7% and accuracy of 82.1%.The sensitivity of Low-grade UC and High-grade UC is 62.7% and 74% respectively indicating that High-grade UC shed malignant cells more readily than Low-grade UC.

The false negative and false positive cases in this study, can be minimized by proper collection of urine samples, proper fixation and staining methods, screening, interpretation and obtaining previous history of bladder stones, chemotherapy/radiotherapy and recent urinary tract instrumentation and surgery.

Cytological examination of urine specimen is a simple, safe and inexpensive method for detection of bladder cancers.

TABLES

Table – 1

Sex distribution of all cases

Sl. No.	Sex	No. of cases	Percentage
1	Male	125	78%
2	Female	35	22%
	Total	**160**	**100%**

Diagram - 1

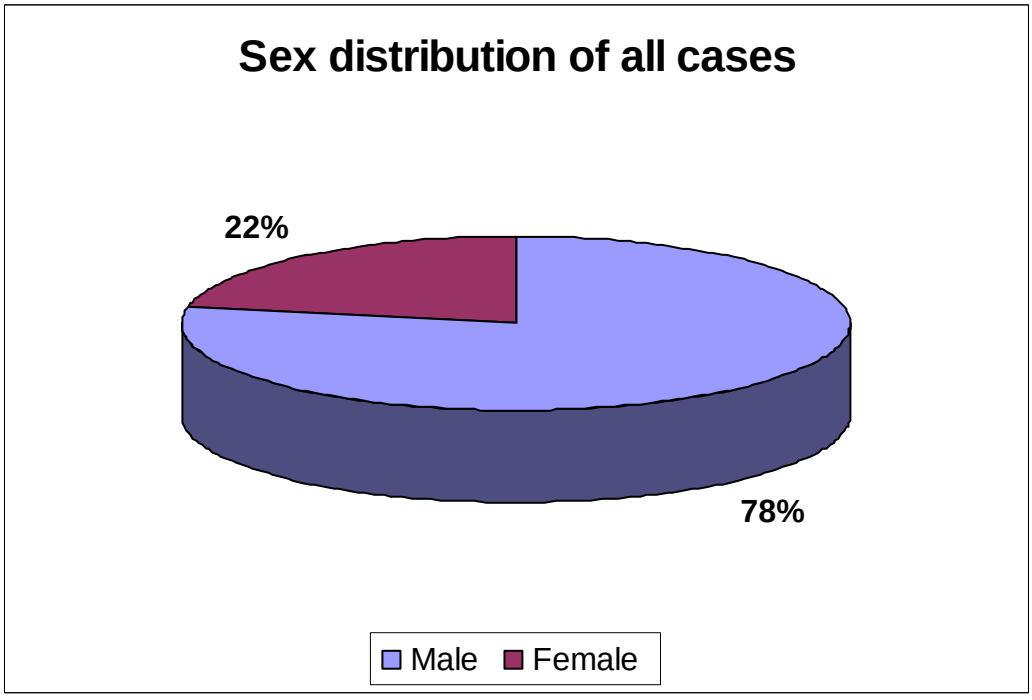

Table – 2

Age and sex distribution of all cases

Age Group	No. of cases		Total %
	Male	Female	
11-20	1	2	3 (1.9%)
21-30	3	4	7 (4.4%)
31-40	13	13	26 (16.2%)
41-50	20	5	25 (15.6%)
51-60	44	3	47 (29.4%)
61-70	37	4	41 (25.6%)
71-80	9	1	10 (6.2%)
81-90	1	-	1 (0.6%)
Total	**125**	**35**	**160 (100%)**

Diagram - 2

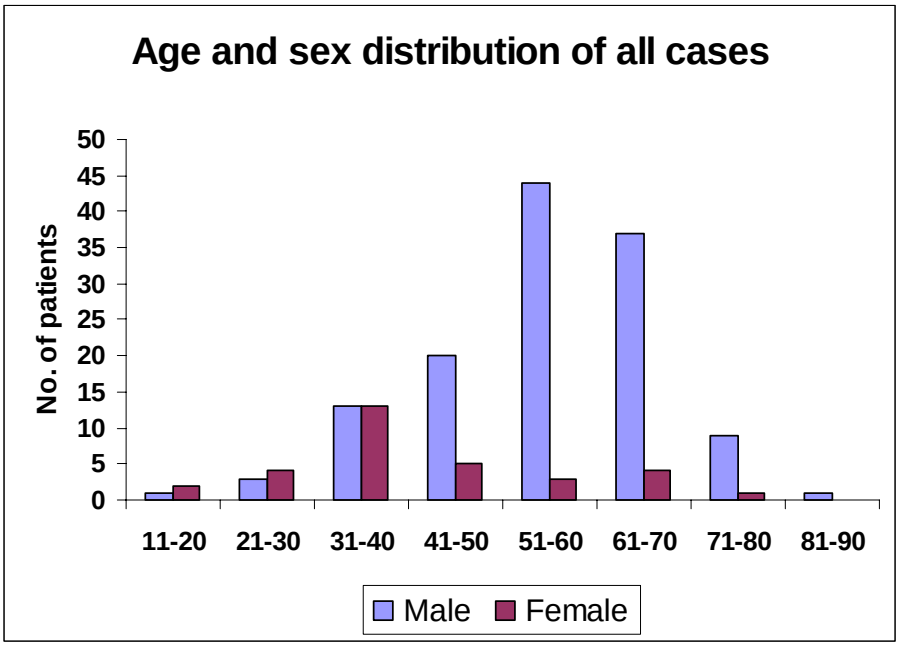

Table – 3

Distribution of Smoking habit

Group	Male		Female	
	No of cases	%	No of cases	%
Smoker	91	72.8%	-	-
Non-smoker	34	27.2%	35	100%
Total	**125**	**100%**	**35**	**100%**

Diagram - 3

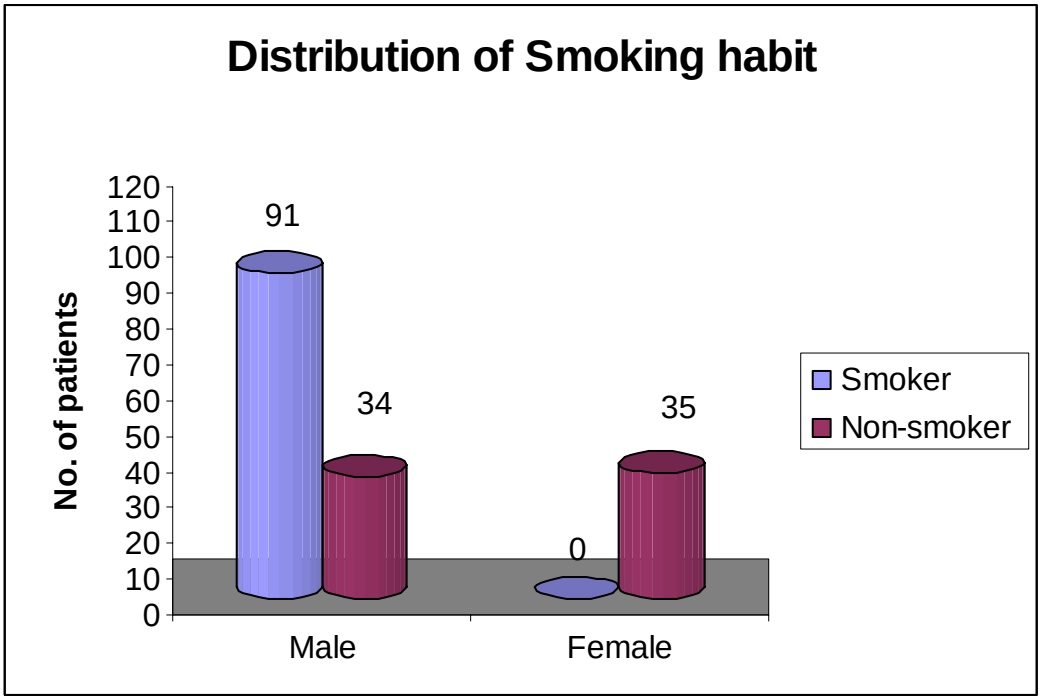

Table – 4

Clinical symptoms of all cases

Sl. No.	Clinical symptoms	No. of cases
1	Hematuria	53
2	Dysuria	69
3	Frequency of micturition	47

Diagram – 4

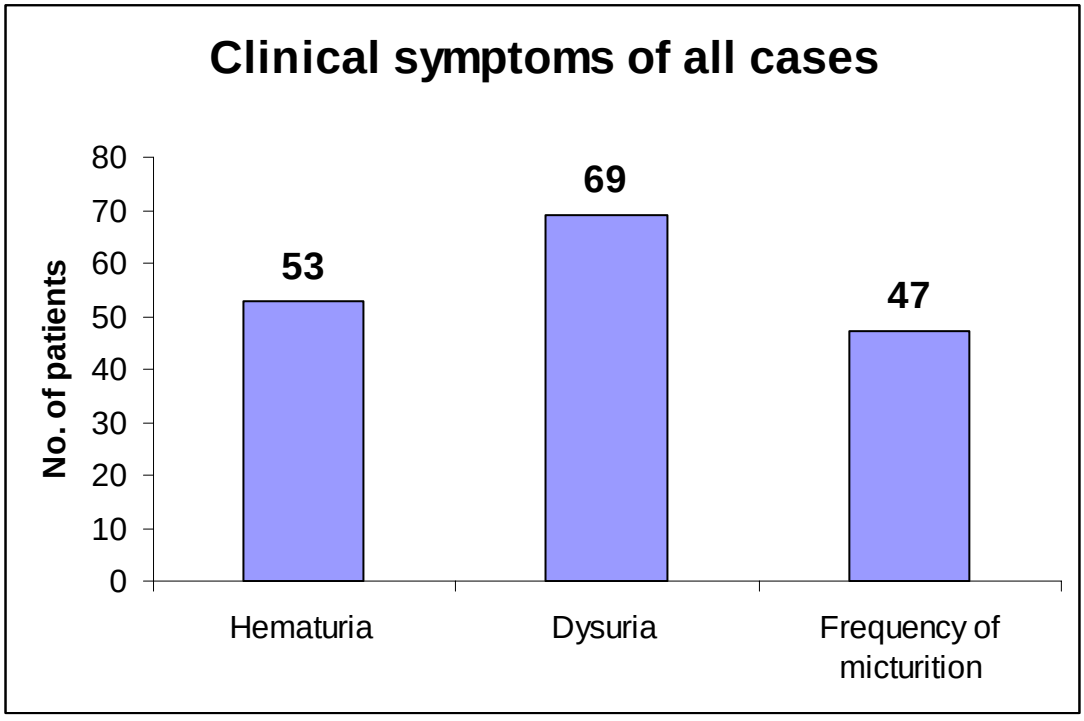

Table – 5

Past history of illness

Sl. No.	Past History	No. of cases		Total
		Male	Female	
1	Bladder stones	20	7	27
2	Recent surgery in the bladder	13	1	14
3	Bladder tumors	12	1	13
4	Tuberculosis	1	-	1
5	Pelvic irradiation	11	1	12

Diagram - 5

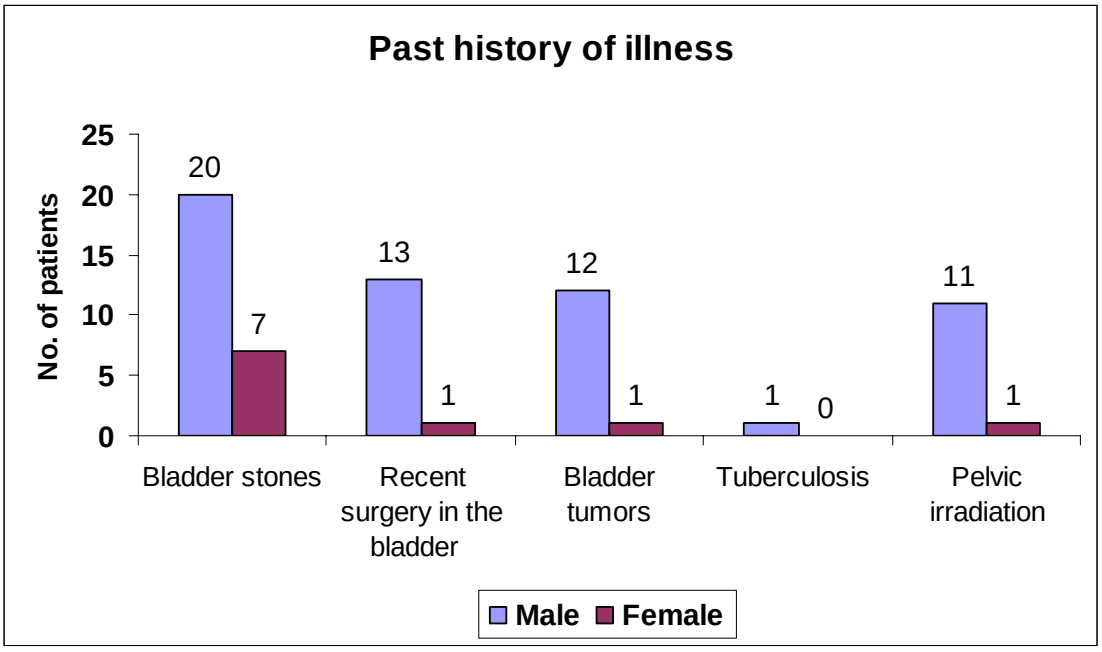

Table – 6

Cytological diagnosis in 160 urinary samples

Sl. No.	Lesion	No. of cases		Total %
		Male	Female	
1	Normal	32	12	44 (27.5%)
2	Non specific cystitis	38	15	53 (33.1%)
3	Reactive	13	3	16 (10%)
4	Low grade UC	3	1	4(2.5%)
5	High grade UC	39	2	41(25.6%)
6	SCC	-	1	1(0.6%)
7	Adeno carcinoma	1	-	1(0.6%)
	Total	**126**	**34**	**160 (100%)**

Diagram - 6

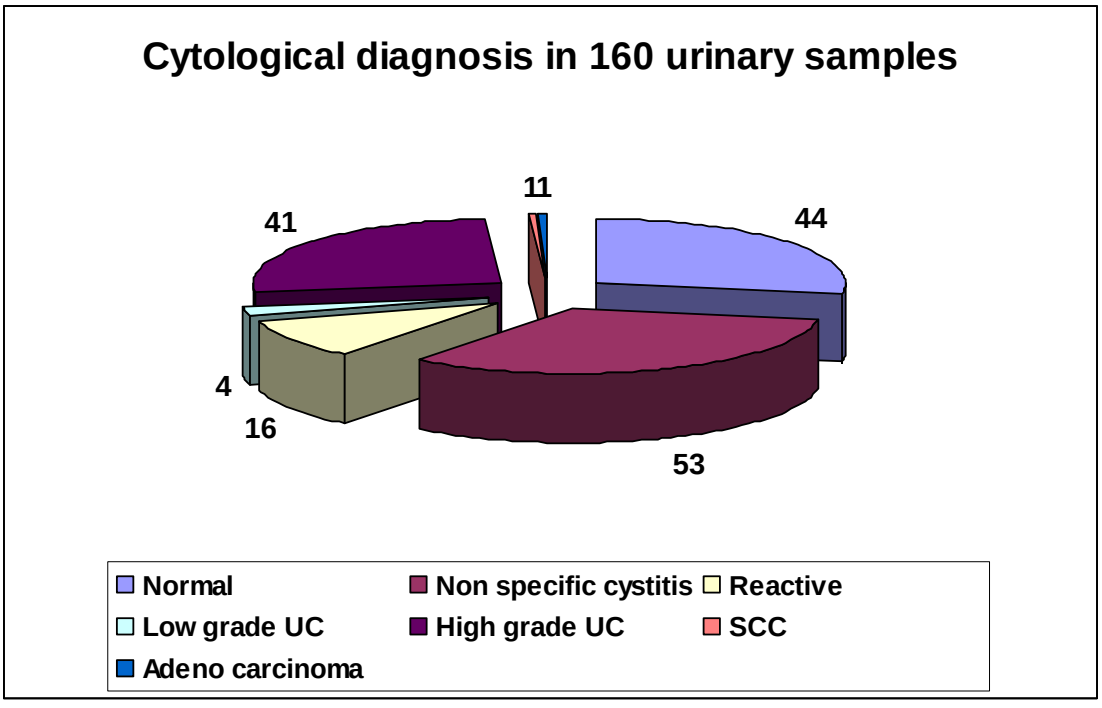

Table – 7

Histological diagnosis of bladder biopsy in 84 samples

Sl. No.	Lesion	No. of cases		Total %
		Male	Female	
1	Normal	7	-	7 (8.3%)
2	Non specific cystitis	5	12	17 (20.2%)
3	TB cystitis	3	0	3 (3.6%)
4	Schistosomiasis	1	0	1(1.2%)
5	Leiomyoma	-	1	1(1.2%)
6	Transitional cell papilloma	1	-	1(1.2%)
7	Urothelial carcinoma(UC)			
	Papillary Grade I (Low grade)	5	1	
	Invasive Grade II (High grade)	31	2	52(61.9%)
	Invasive Grade III (High grade)	12	1	
8	SCC	-	1	1(1.2%)
9	Adenocarcinoma	1	-	1(1.2%)
	Total	**66 (78.6%)**	**18 (21.4%)**	**84 (100%)**

Diagram – 7

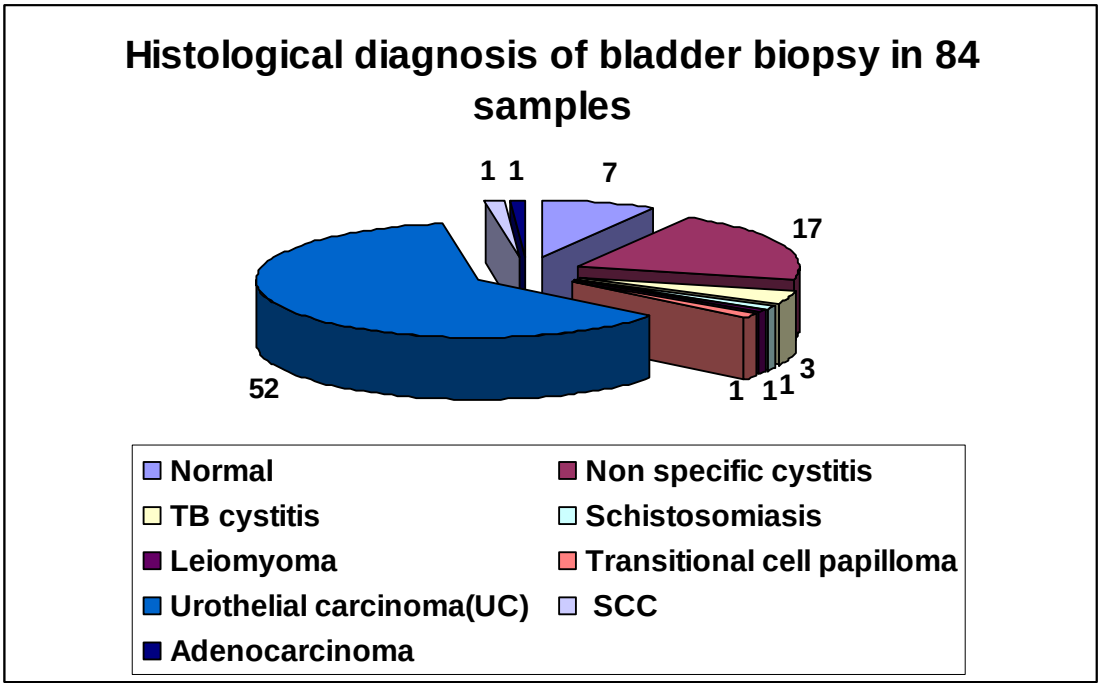

Diagram – 8

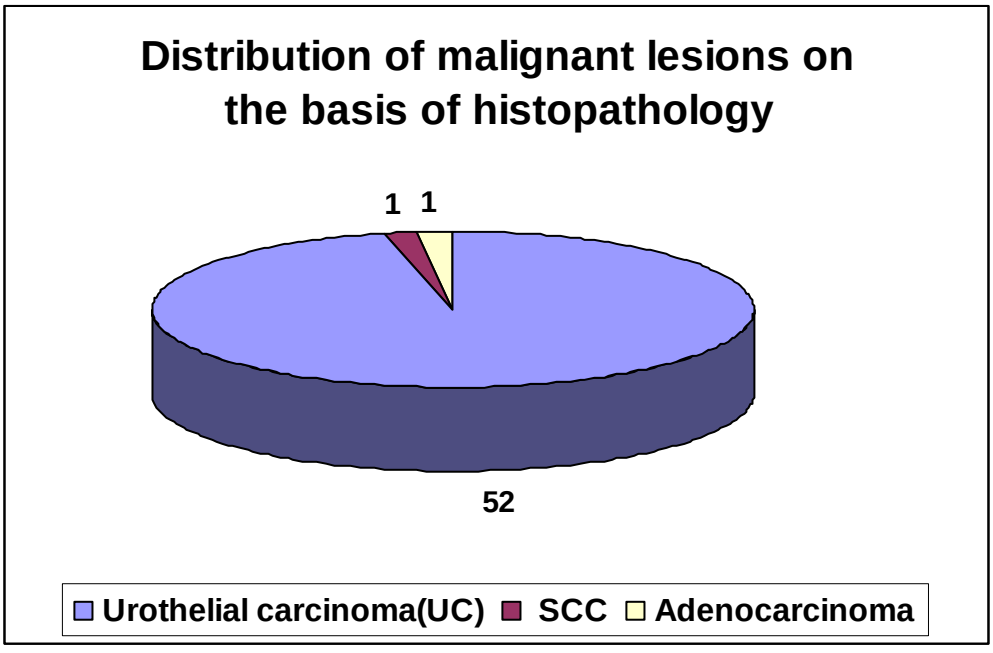

Diagram - 9

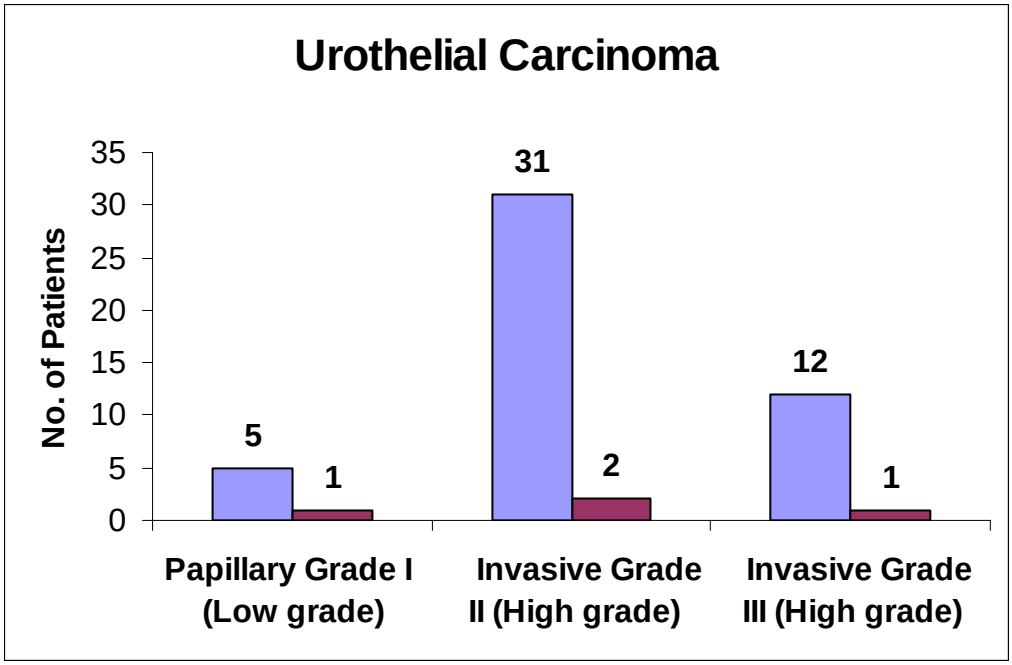
Urothelial Carcinoma
No. of Patients
35
30
25
20
15
10
5
0
5
1
31
2
12
1
Papillary Grade I (Low grade)
Invasive Grade II (High grade)
Invasive Grade III (High grade)

Table – 8 Comparison of cytologic diagnosis of urine with histopathological diagnosis

Cytological diagnosis	Final histopathological diagnosis																						
	Normal		TB cystitis		Schistosomiasis		Non specific cystitis		Leiomyoma		Transitional cell papilloma		Urothelial carcinoma						Squamous cell carcinoma		Adeno carcinoma		Total
													I		II		III						
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
Normal	1		1				3	4					1		6	1	2	1					20
Nonspecific cystitis	1		1				1	7					1		2								13
Reactive	4		1		1		1	1		1	1												10
Low grade UC													3	1									4
High grade UC	1														23	1	10						35
SCC																				1			1
Adenocarcinoma																					1		1
Total	7		3		1		5	12		1	1		5	1	31	2	12	1		1	1		84

Table - 9

Efficacy of urine cytology report in present study

True Positive	False Positive	True Negative	False Negative	Sensitivity	Specificity	Accuracy	PPV	NPV
40	1	29	14	74.1	96.7	82.1	97.6	67.4

Diagram - 10

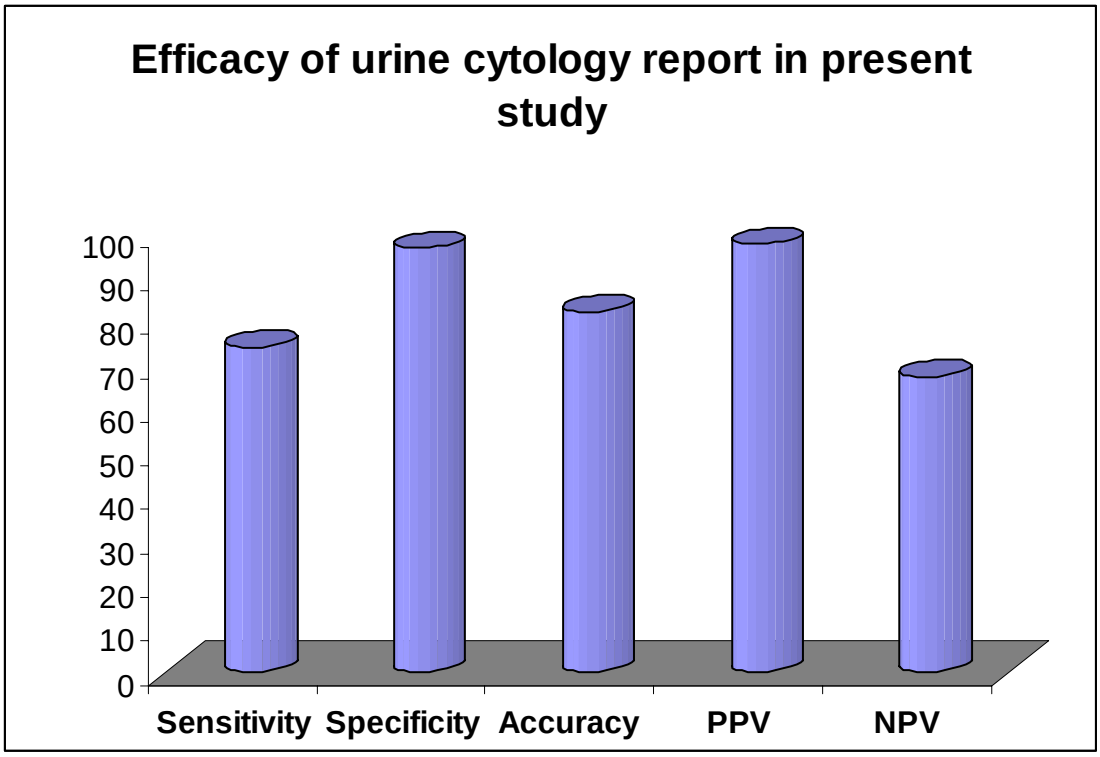

Table – 10

Comparative Study

Study	Overall sensitivity of bladder cancer
Geisse LJ et al (1978) [20]	97%
William M. Murphy et al (1983)[42]	92%
Tanaka K et al (1990)[55]	50%
Leopald G et al (1995)[36]	78-94.2%
Present study 2008	74.1%

Table-11

Correlative study for sensitivity of Low-grade UC and High-grade UC

Study	Low-grade UC	High-grade UC
Murphy WM et al 1984	0-73%	95%
Present study 2008	62.7%	74%

Table -12

Proportion of positive malignant cases seen in cytology in the present study

Histopathological Diagnosis	Cytological diagnosis		
	Total No. Cases	Negative	Positive
Urothelial carcinoma			
Papillary Grade I	6	2	4 (66.7%)
Invasive Grade II	33	9	24 (72.7%)
Invasive Grade III	13	3	10 (76.9%)
Squamous cell carcinoma	1	0	1 (100%)
Adenocarcinoma	1	0	1 (100%)
Total	**54**	**14**	**40**

Table -13 Correlative study for proportion of positive malignant cases detected by cytology

Study	Urothelial carcinoma			SCC	Adeno carcinoma
	I	II	III		
Kern W H 1997	31%	44%	72%	75%	63%
Present study 2008	66.7%	72.7%	76.9%	100%	100%

CHARACTERISTICS OF UROTHELIAL CARCINOMAS

Superficial	Invasive
Approx.66% of tumours	Approx. 25% of tumours
Papillary	Sessile
Low grade	High grade
Low stage	High stage
High recurrence rate	N/A
Low progression rate	High progression rate
Low mortality	High mortality

Cytological features of reactive urothelial cells, low-grade UC and high-grade UC

Cytologic feature	Reactive	Low-grade UC	High-grade UC
Cell arrangements	Papillary aggregates	Papillary and loose clusters	Isolated cells and loose clusters
Cell size	Increased	Increased and uniform	Increased and pleomorphic
Cell number	Variable	Often numerous	Usually numerous
Cytoplasm	Vacuolated	Homogenous	Vacuolated
N/C ratio	Normal/Increased	Increased	Increased
Position of nucleus	Eccentric	Eccentric	Eccentric
Nuclear size	Uniform	Enlarged	Variable
Nuclear contour	Smooth	Irregular,notches and grooves	Markedly irregular
Nuclear chromatin	Fine , regular	Fine, irregular	Coarse, irregular
Nucleoli	Often large	Small/absent	Variable

Modified from Murphy et al.

ANNEXURE I

PAP STAINING

Materials required

- Harris hematoxylin was prepared using Pottasium alum and mercuric oxide and filtered into a dark bottle for storage. The working solution was replaced every 1 to 3 weeks, depending on the number of slides being stained.
- OG 6

  Orange G 1.0% solution in 95% Alcohol - 100 ml

  Phosphotungstic acid - 0.015 gm
- EA 36

  Light green SF Yellowish-o.14% in 95% Alcohol - 45ml

  Bismark brown Y -0.5% in 95% Alcohol - 10 ml

  Eosin yellow -0.55 % in 95% Alcohol - 45 ml

  Phosphotungstic acid - 0.2 gm

  Lithium carbonate, saturated aqueous solution - 1 drop

PROCEDURE

1. Slides were transferred directly from the fixative, without drying,to 95 % Alcohol and brought down through 70 and 50 % alcohols to distilled water .
2. Slides were stained in Harris hematoxylin for 5 minutes.
3. And gently rinsed briefly in distilled water.
4. They were dipped in 0.25% Hcl in 50% Ethanol (Acid alcohol) about 6 times for 20 seconds.
5. And placed in running tap water for 6 minutes.
6. They were rinsed in distilled water and run through 70%, 80% to 95% Alcohol.
7. And stained in OG 6 for 3 minutes.
8. Rinsed in two changes of 95% Alcohol.
9. Stained in EA 36 for 3 minutes.
10. Rinsed in three changes of 95% Alcohol.

Dehydrated in Absolute alcohol, followed by equal parts of Absolute alcohol and Xylol, cleared in Xylol and mounted.

MODIFIED HEMATOXYLIN AND EOSIN STAINING

Preparation of Ehrlich's haematoxylin solution

Haematoxylin	2g
Absolute alcohol	100 ml
Glycerin	100 ml
Distilled water	100 ml
Glaciel acetic acid	10 ml
Potassium alum	15 g approx.

The haematoxylin is dissolved in the alcohol and the other chemicals are added.

Eosin Y - 1% solution in distilled water

PROCEDURE

1. After alcohol fixation, the slides were stained with Ehrlich's haematoxylin for 2 hrs.
2. Then washed well in running tap water until smears "blue" for 5 mins or less.
3. Then differentiated in 1 % acid alcohol for 5-10 sec.

4. Then washed well in tap water until smears are again "blue" for 5 mins or less.
5. Smears were then stained in 1 % eosin Y for 10 min.
6. Again washed with water for 1-5 min.
7. Then dehydrated through alcohol, cleared and mounted.

ANNEXURE-II

PROFORMA

CASE NO :

URINARY CYTOPATHOLOGICAL FINDINGS IN BLADDER LESIONS

NAME :

AGE : SEX :

IP/ OP NO. : UNIT : WARD :

ADDRESS :

CYTO NO : HPE NO.:

SMOKING HISTORY :

PRESENT HISTORY

HEMATURIA :

DYSURIA :

FREQUENCY OF MICTURITION :

PAST HISTORY

H/O INSTRUMENTATION INCLUDING CATHETERIZATION :

H/O BLADDER STONES :

H/O BLADDER TUMOURS :

H/O BLADDER OUTLET OBSTRUCTION INCLUDING BPH :

H/O ANY SURGERY DONE TRANSURETHRALLY :

H/O EXPOSURE TO ANY DRUGS INCLUDING CHEMOTHERAPY :

H/O PELVIC IRRADIATION :

H/O TB/ SCHISTOSOMIASIS / ANY VIRAL INFECTIONS :

CLINICAL DIAGNOSIS :

INVESTIGATIONS:

HEMATOLOGICAL:

RADIOLOGICAL :

CYSTOSCOPY :

URINE CYTOLOGY:

SQUAMOUS CELLS:

COLUMNAR CELLS:

UROTHELIAL CELLS:

ARRANGEMENT OF CELLS:
CELLS :
N/C RATIO :
NUCLEUS :
NUCLEAR MEMBRANE :
CHROMATIN :
NUCLEOLI :
CYTOPLASM :
MITOTIC FIGURES :
BACK GROUND :

INFLAMMATORY CELLS:
NEUTROPHILS/EOSINOPHILS/LYMPHOCYTES/HISTIOCYTES

ORGANISMS:

OTHERS :

SPECIAL STAINS, IF ANY:

CYTOLOGICAL DIAGNOSIS:

BIOPSY STUDIES, IF ANY:

MARKER STUDIES ,IF ANY:

FINAL DIAGNOSIS:

ANNEXURE – III

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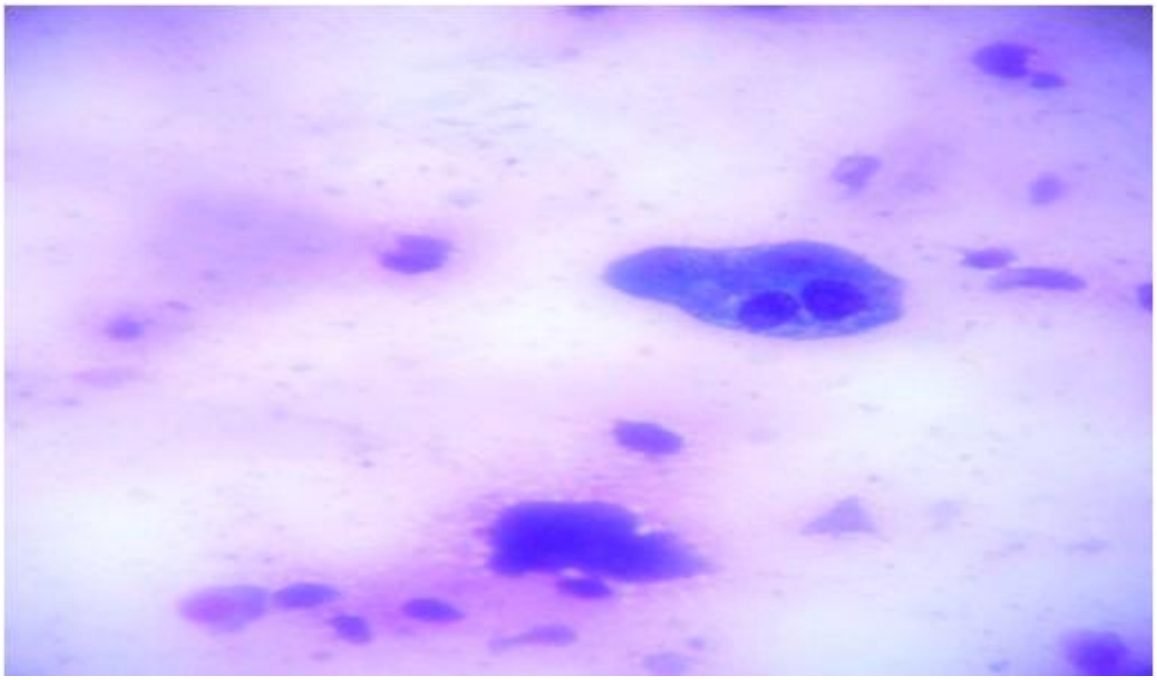

Fig.1 Superficial urothelial cell and basal cells in the normal urine cytology smear. H and E stain x 450 (M 145/07)

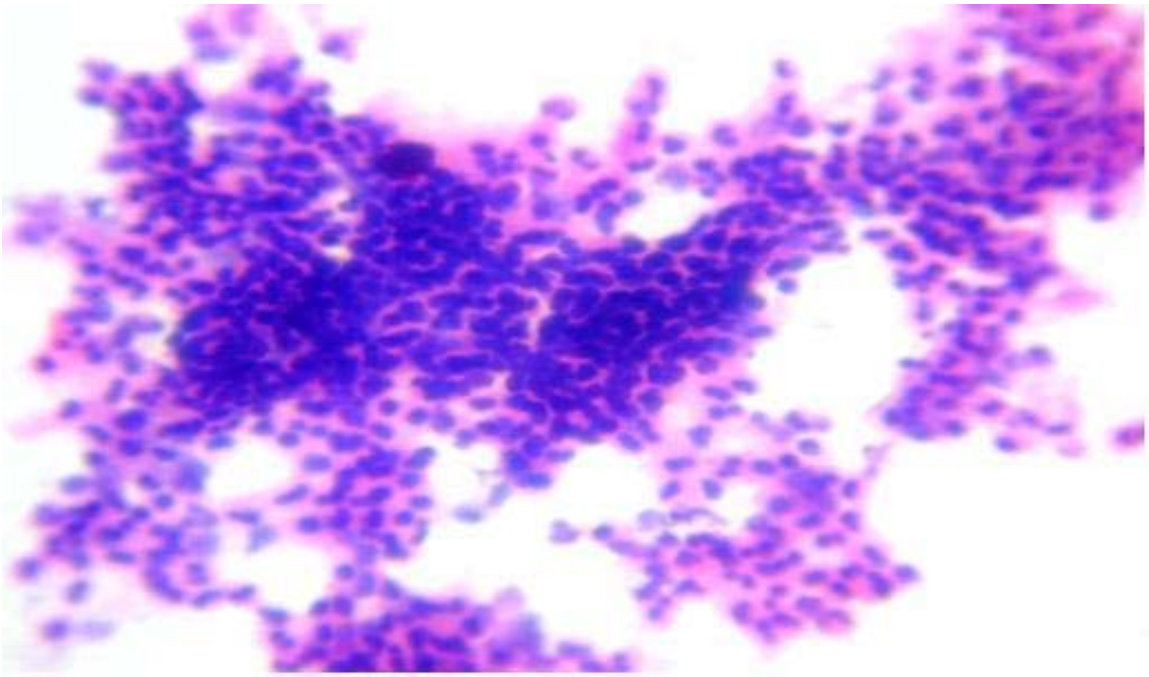

Fig.2 Numerous neutrophils, histiocytes and a few lymphocytes along with the urothelial cells in non specific cystitis. Pap stain x 450 (1055/07)

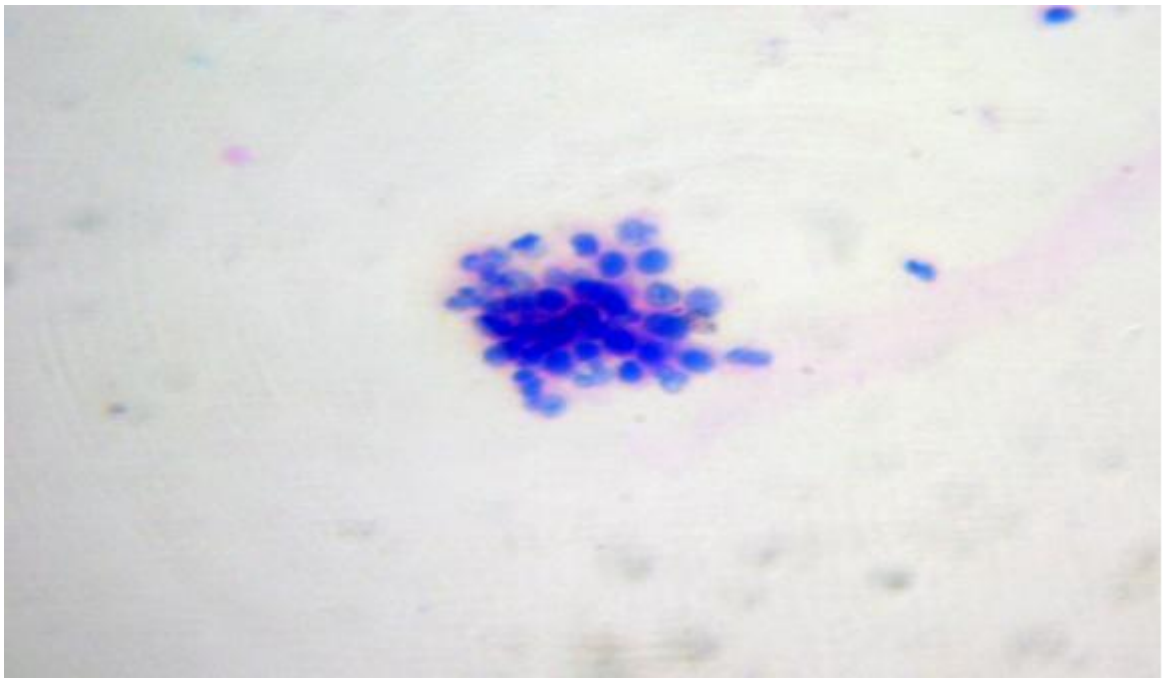

Fig.3 Low grade urothelial carcinoma; Cells arranged in loose papillary clusters with increased nuclear/cytoplasmic ratio. Pap stain x 450 (1368/07)

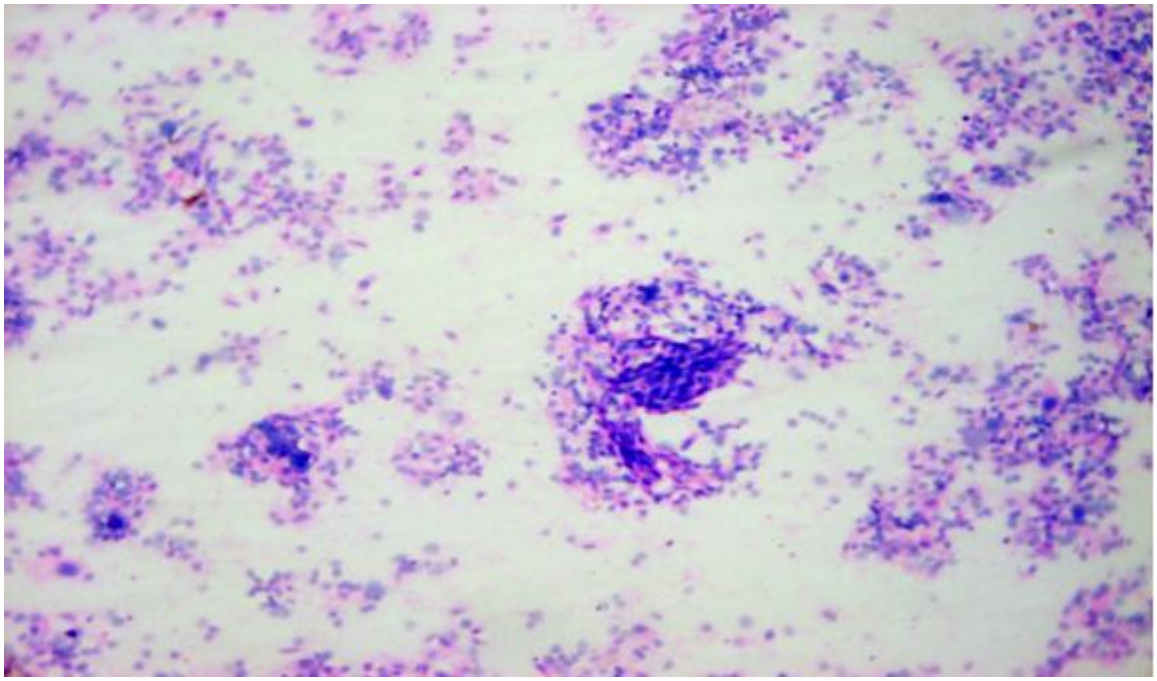

Fig.4 High grade urothelial carcinoma; Cellular smear in which cells are arranged in loose clusters as well as isolated cells. Pap stain x100 (M688/07)

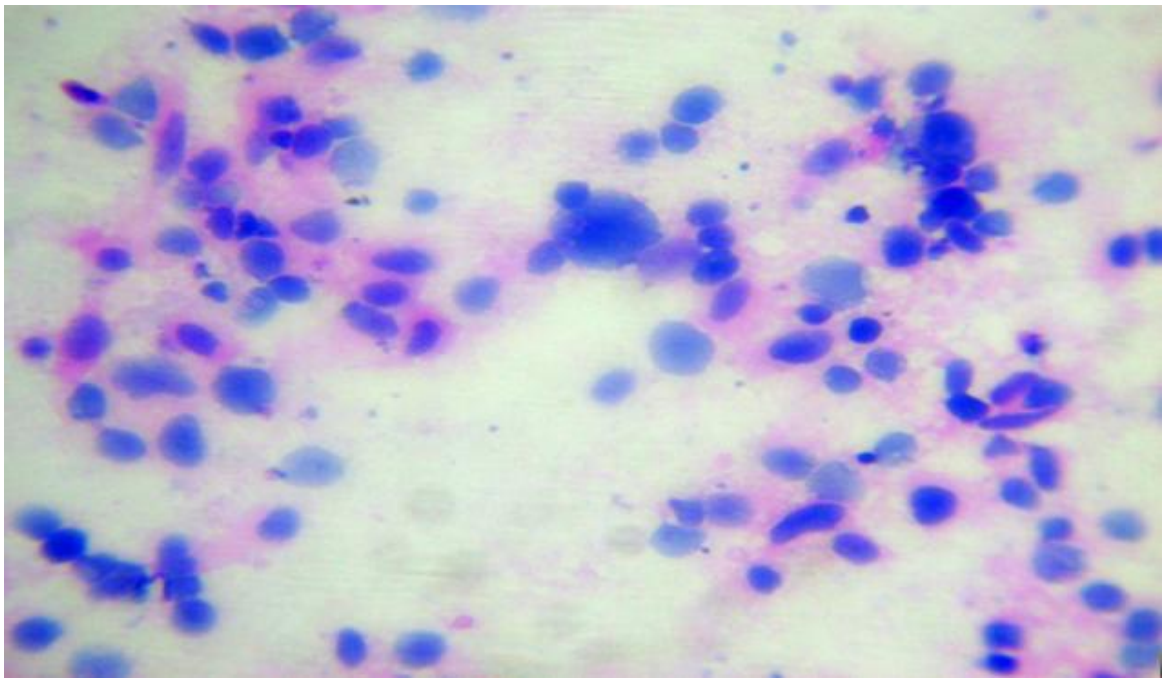

Fig.5 Squamous cell carcinoma; Cellular smear with large pleomorphic cells with hyperchromatic nuclei and eosinophilic cytoplasm. H and E stain x 450 (803/07)

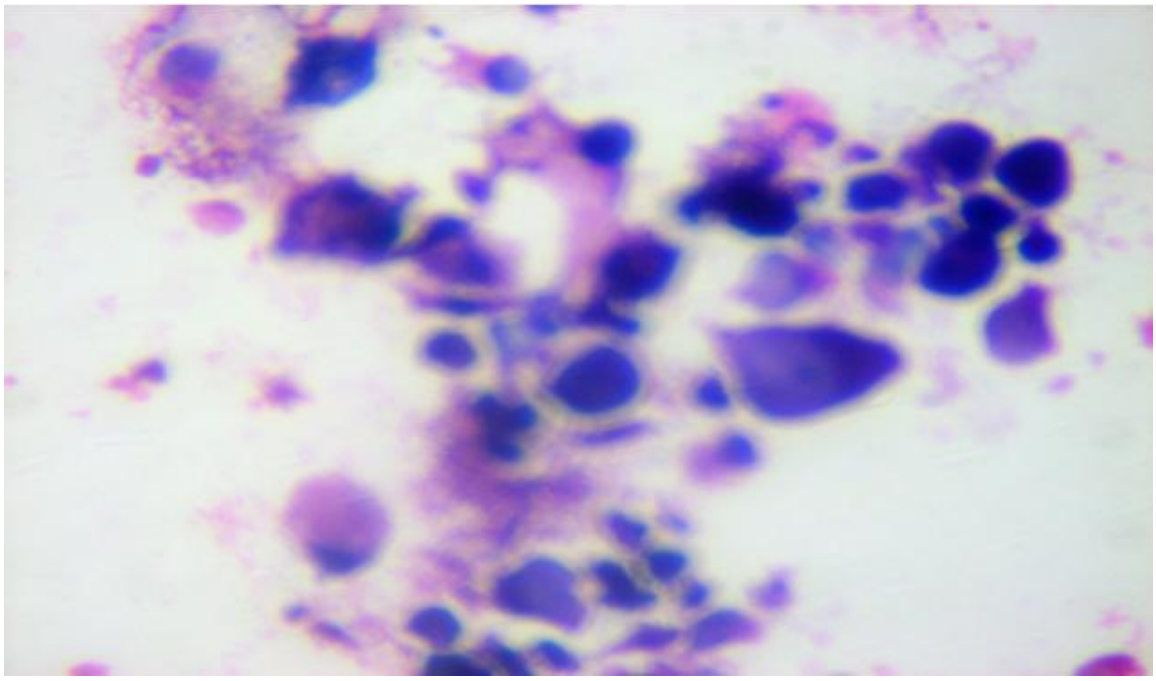

Fig.6 Adenocarcinoma; Pleomorphic tumour cells arranged in acinar pattern. H and E stain x 1000 (M214/07)

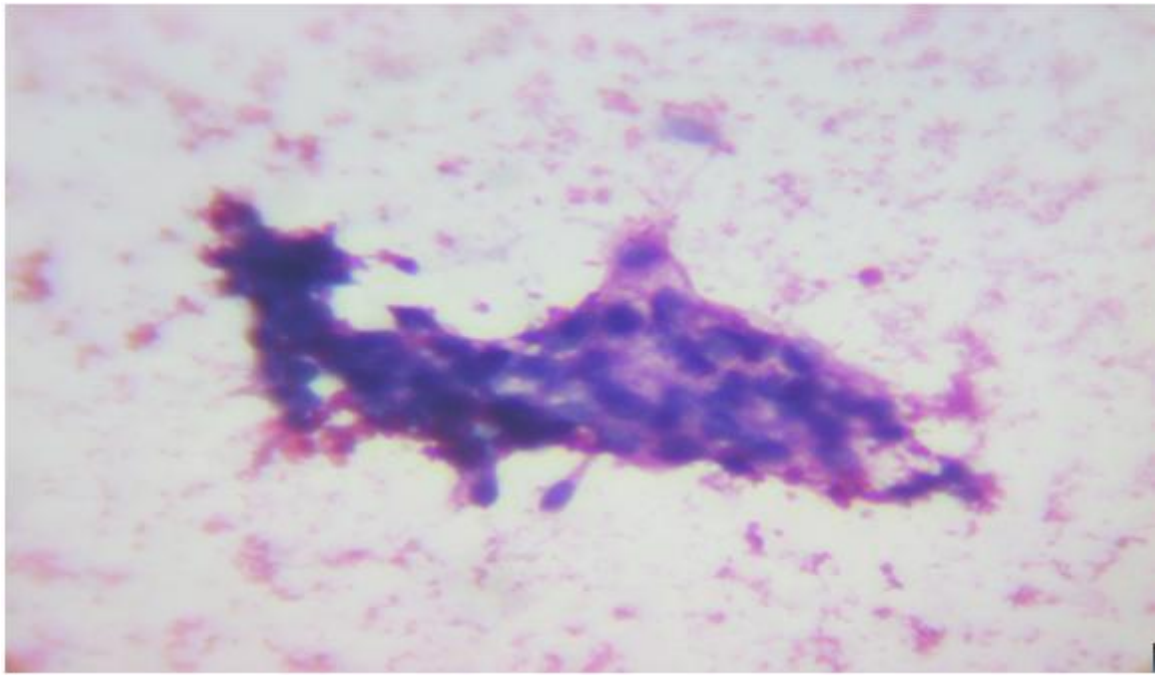

Fig.7 Calculous artifact; Cells arranged in papillary clusters with ragged borders and abnormal nuclei. Pap stain x 450 (M980/07)

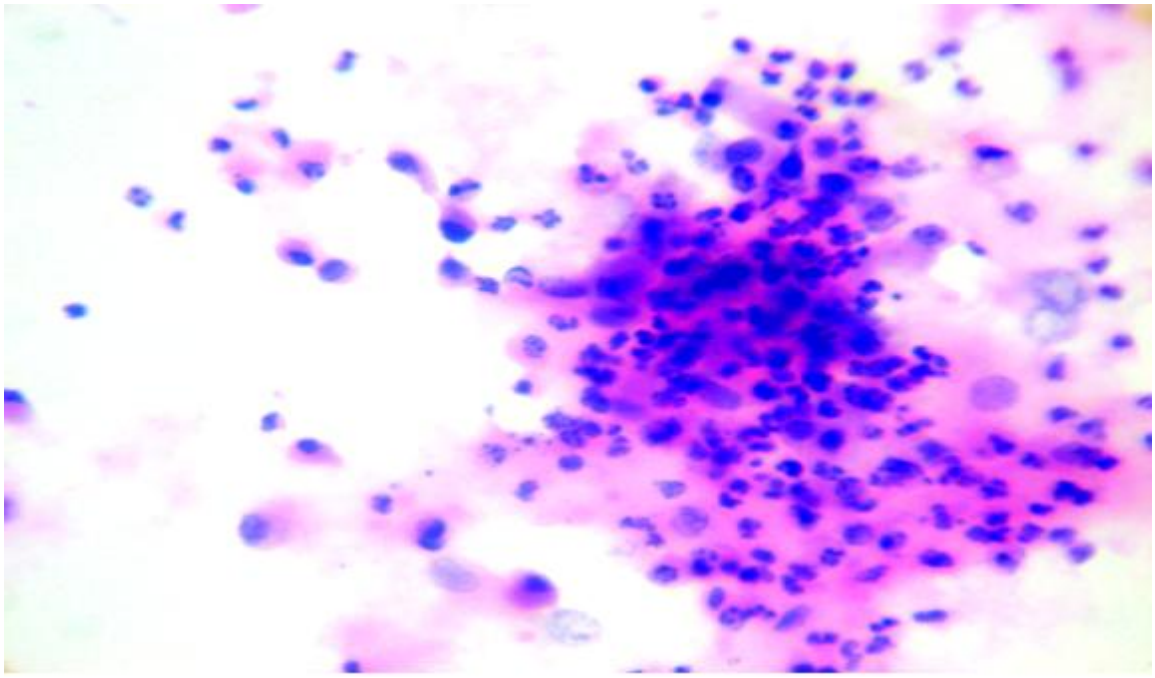

Fig.8 Radiation induced change; Irregularly enlarged cells with irregular, hyperchromatic nuclei and karyorrhexis. Pap stain x 1000 (1220/07)

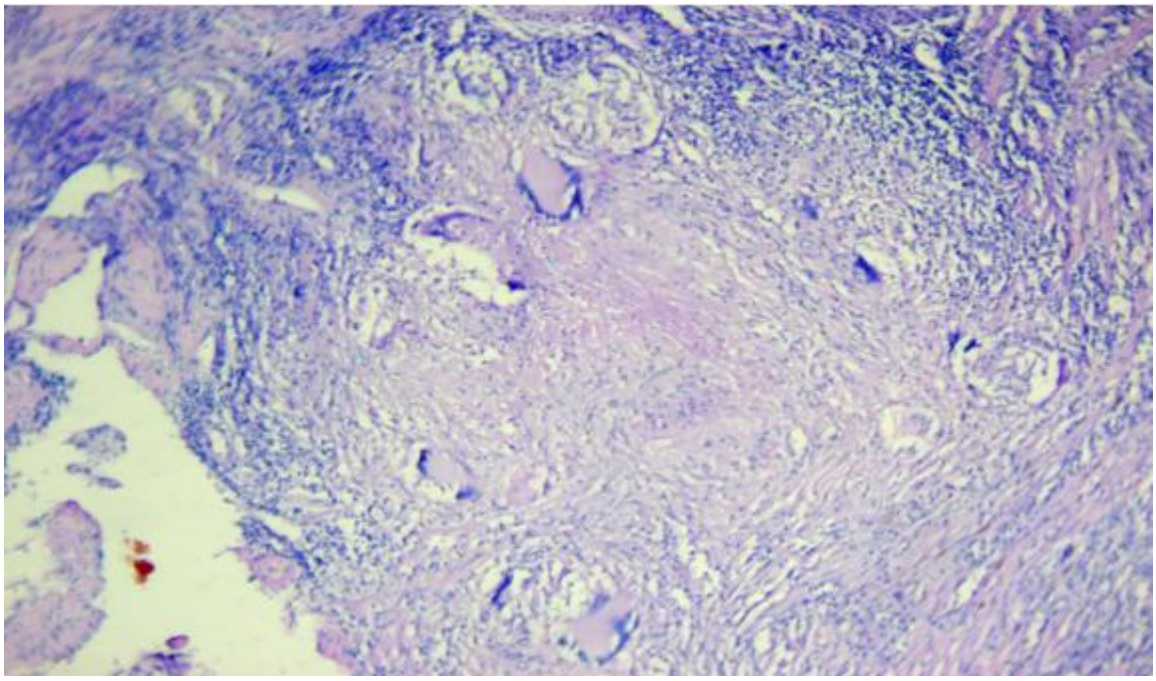

Fig.9 Tuberculous cystitis; Bladder wall showing numerous epithelioid granulomas and Langhans' giant cells. H and E stain x 450 (4604/07)

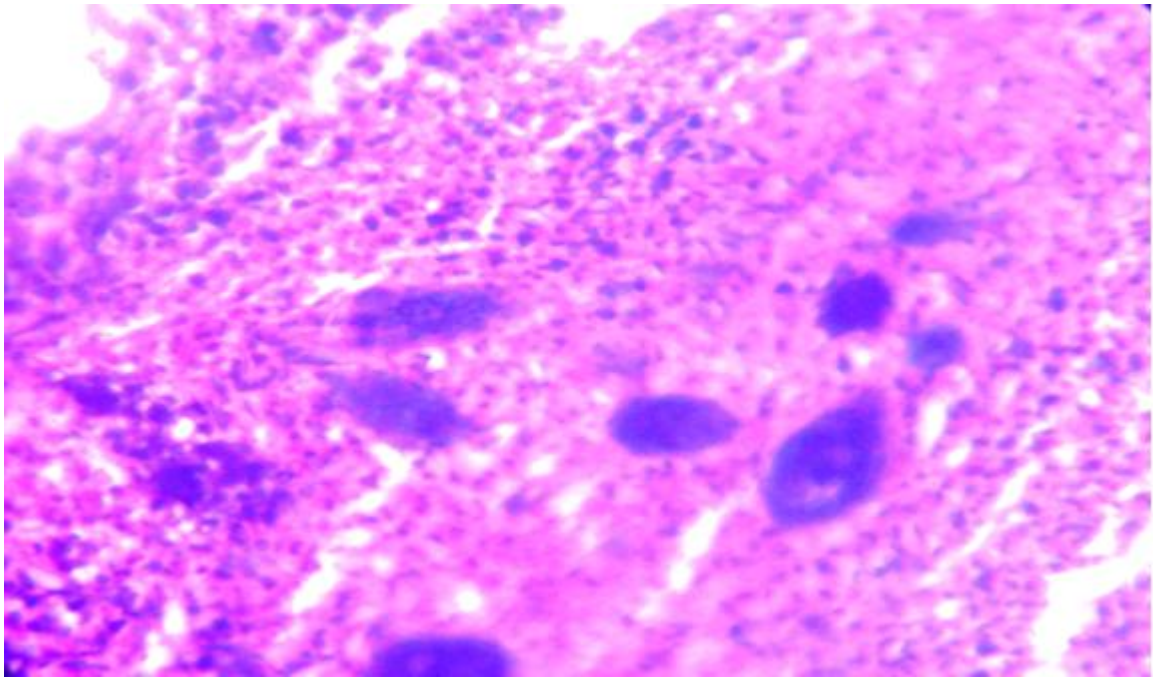

Fig.10 Schistosomiasis; Numerous schistosoma haematobium ova with terminal spine seen in the submucosa of the bladder. H and E stain x 450 (M2901/07)

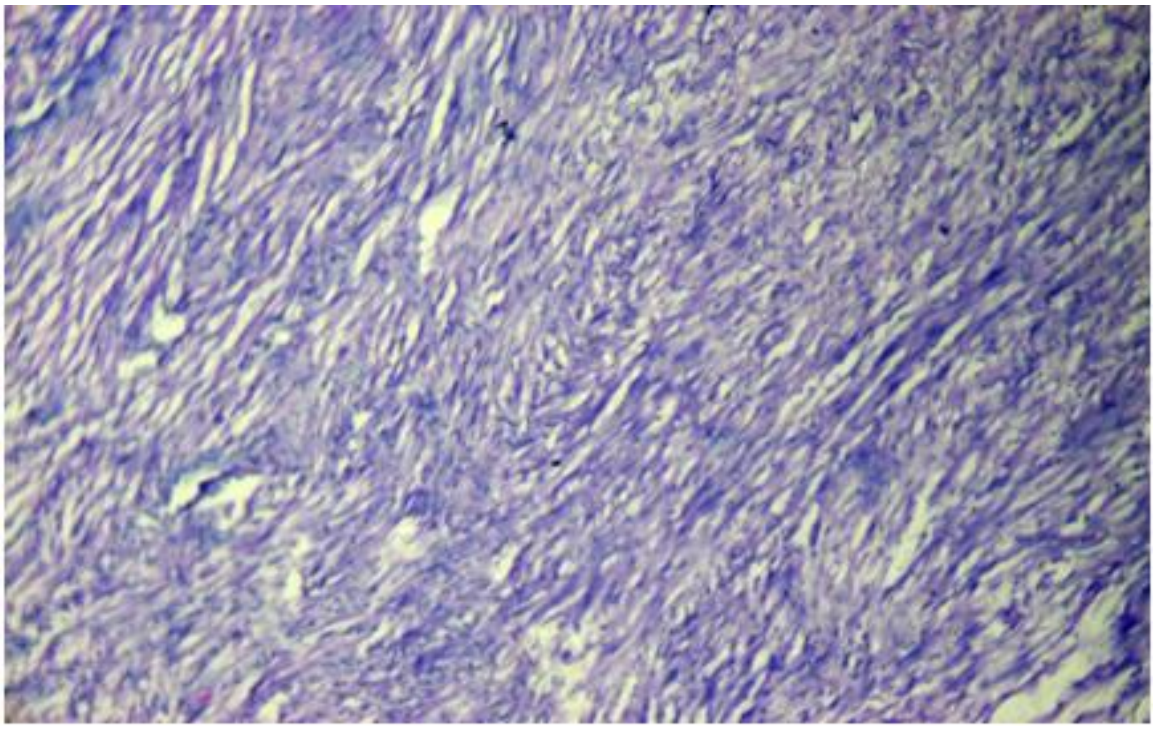

Fig.11 Leiomyoma of the bladder; Tumour composed of spindle cells arranged in fascicles. H and E stain x 450 (M2162/07)

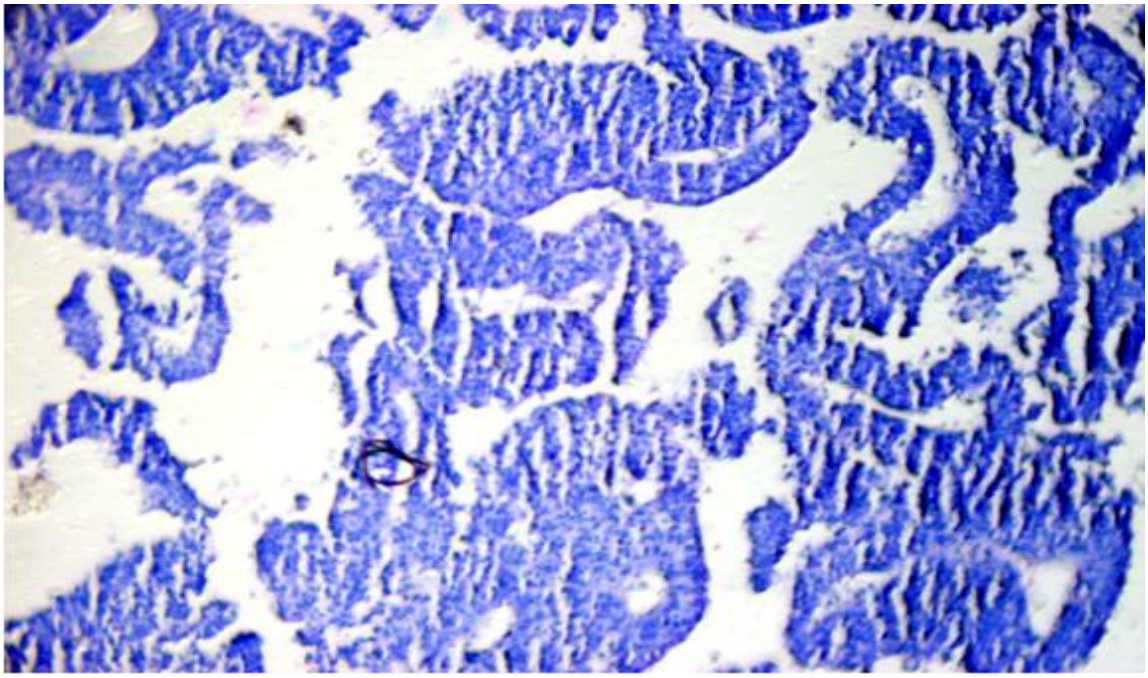

Fig.12 Papillary urothelial carcinoma grade I; Composed of connective tissue stalk covered by well differentiated cells. H and E stain x 450 (4178/07)

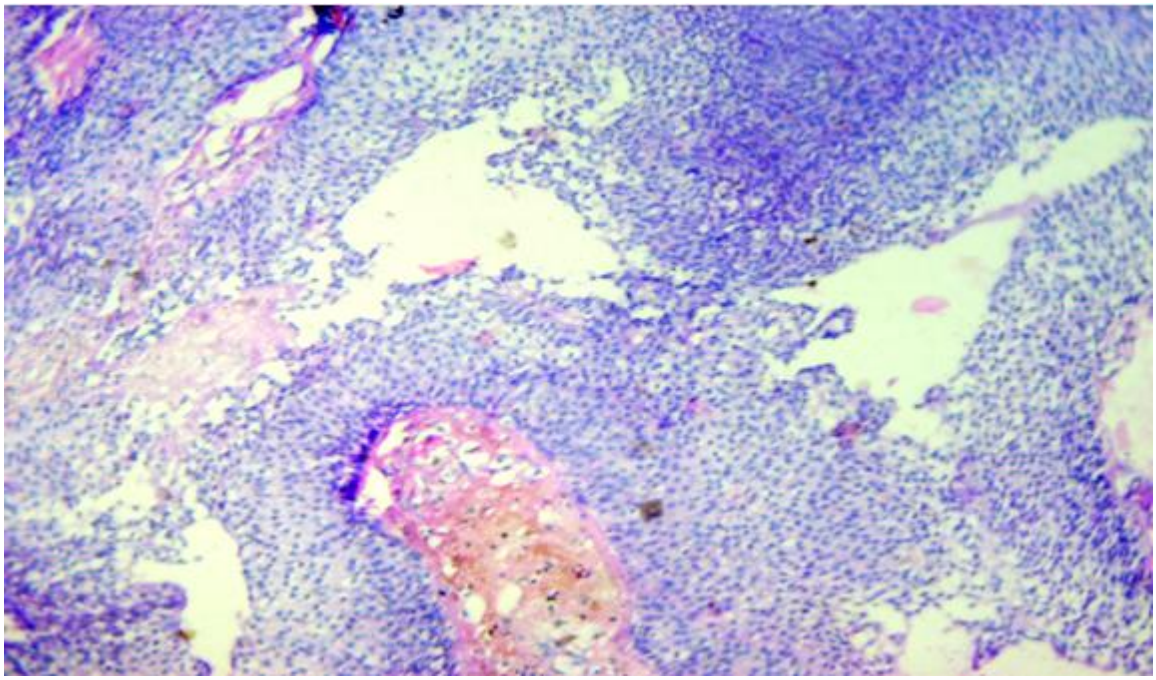

Fig.13 Urothelial carcinoma grade II; Relatively uniform cancer cells separated from each other by bands of connective tissue. H and E stain x 450 (2899/07)

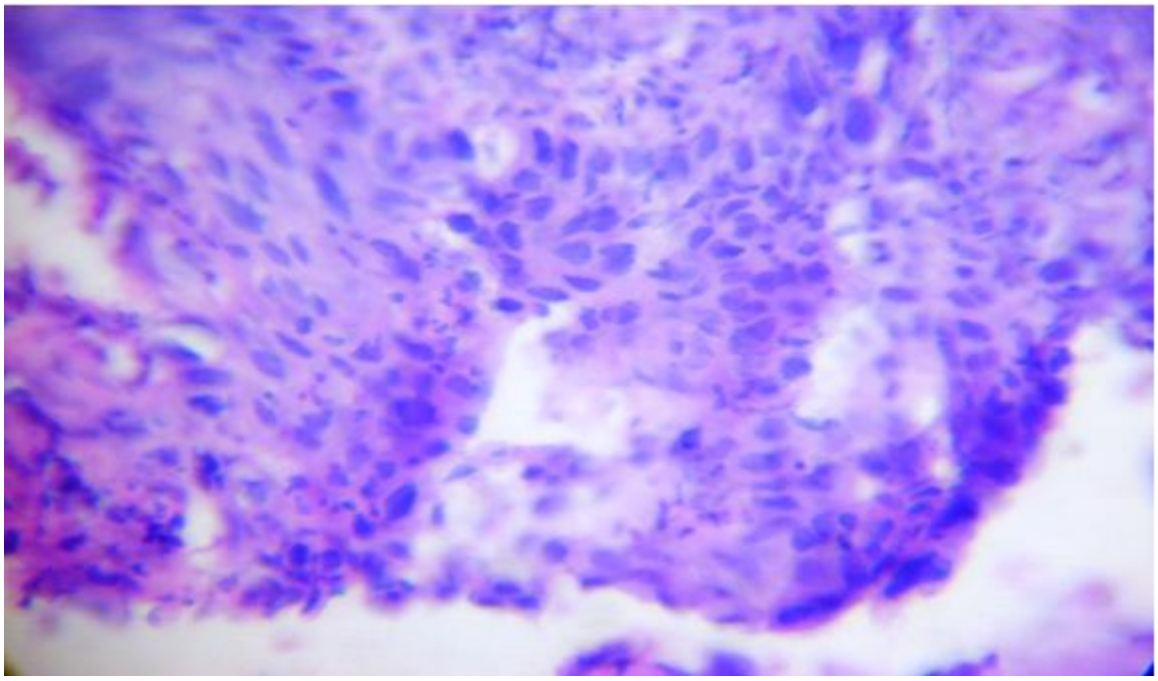

Fig.14 Urothelial carcinoma grade III; Cancer cells with variability in size of cells and marked nuclear abnormalities. H and E stain x 450 (1138/08)

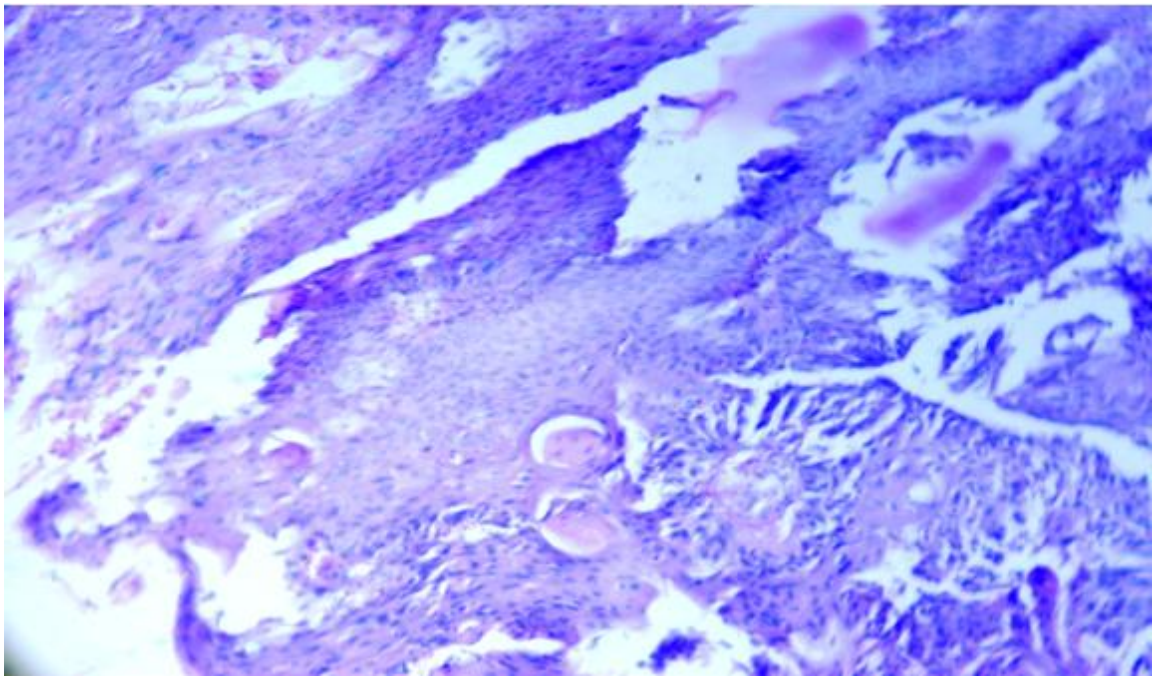

Fig.15 Squamous cell carcinoma; Tumour cells with marked nuclear abnormalities and focal squamous differentiation. H and E stain x 450 (1783/07)

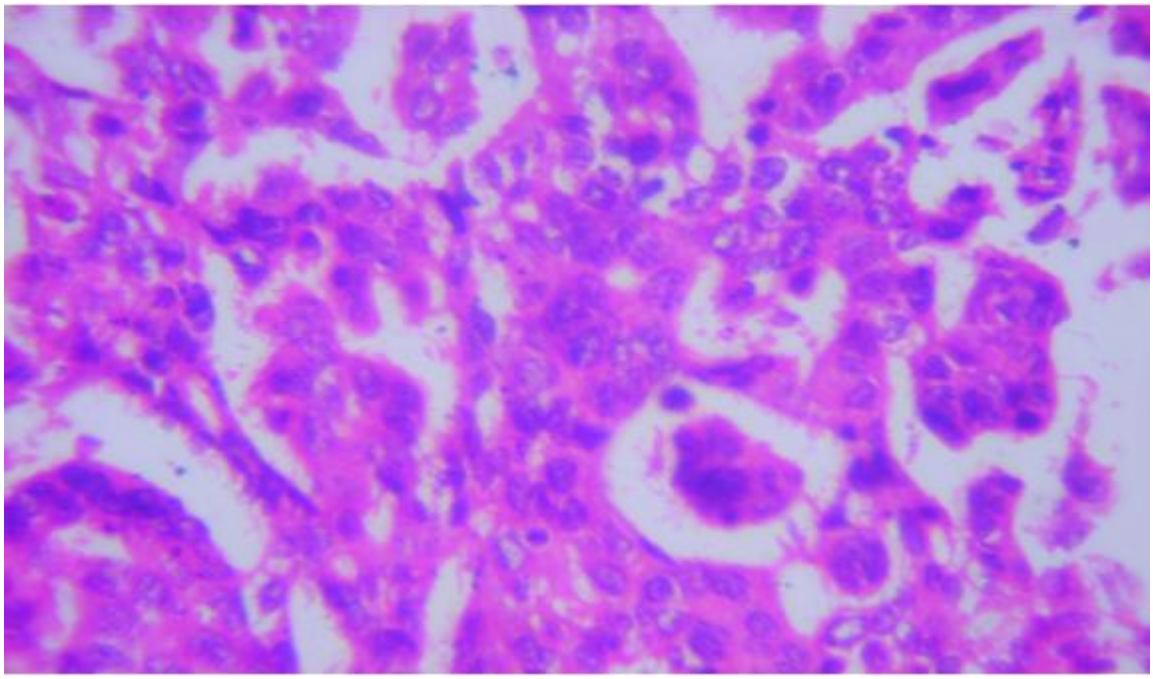

Fig.16 Adenocarcinoma; Pleomorphic tumour cells with vesicular nuclei arranged in glandular pattern. H and E stain x 450 (M270/07)